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(54) Title: A METHOD FOR THE IMPROVEMENT OF TRANSPORT ACROSS ADAPTABLE SEMI-PERMEABLE BARRIERS

(57) Abstract: The invention relates to a method, a kit and a device for controlling the flux of penetrants across an adaptable semi-permeable porous barrier, the method comprising the steps of: preparing a formulation by suspending or dispersing said penetrants in a polar liquid in the form of fluid droplets surrounded by a membrane-like coating of one or several layers, said coating comprising at least two kinds of forms of amphiphilic substances with a tendency to aggregate, said penetrants being able to transport agents through the pores of said barrier or to enable agent permeation through the pores of said barrier after penetrants have entered the pores, selecting a dose amount of said penetrants to be applied on a predetermined area of said barrier to control the flux of said penetrants across said barrier, and applying the selected dose amount of said formulation containing said penetrants onto said area of said porous barrier.

A Method for the Improvement of Transport Across
Adaptable Semi-Permeable Barriers

5 The present invention is in the field of administration of drugs and particularly drug delivery across barriers. It more particularly relates to a method for controlling the flux of penetrants across an adaptable, semi-permeable porous barrier. It further relates to a kit and a patch which both enable the drug to be controllably applied.

10 A porous barrier as used herein is any obstacle comprising pores which are too narrow to let the penetrants diffusively pass. This necessarily implies that the penetrants are bigger than the average diameter of such a pore.

15 Some barriers, such as artificial porous membranes, for example ion-track polycarbonate membranes, may have permanent properties, while others are characterised by a possible change of their properties. Most notably the pore size and more rarely the pore density, may change as a function of the surroundings and/or of the flux of the penetrants through the pores in the barrier. The latter can be found with living tissues which are separated by boundaries with such
20 properties, for example, cells and cell organelles.

The skin is used to further illustrate the basic principle of such a barrier:

25 The maximum barrier properties of the skin reside in the outermost skin region, that is, in the horny layer (*stratum corneum*). This is owing to special chemical and anatomical characteristics of the horny layer, which preclude most efficiently the passage of essentially any material across the skin.

5 In the stratum corneum, 20-30 consecutive layers of the skin cells (chiefly corneocytes) are organised into columns. These columns are oriented perpendicular to the skin surface, permitting the cells from adjacent columns to overlap laterally and forcing the cells from one layer to be overlaid and packed densely. Intercellular junctions in the horny layer, moreover, are tightly sealed with specialised lipids, chiefly ceramides, which abound in the skin. The skin lipids are also predominantly well packed: typically, they form lipid multilamellae, which are coupled covalently to the neighbouring cell (envelope) membranes. Individual multilamellar stacks that run parallel to the cells surface are joined together with the less well ordered lipid domains. In such domains, the non-ceramide lipids (fatty acids, cholesteryl-sulphate, etc.) prevail.

15 The skin lipid tendency to self-arrange into densely packed, multilamellar structures is enhanced or even driven, by the hydration or certain ion (e.g. Ca^{2+}) concentration gradients in the skin. This may explain why similar lipid organisation is not observed elsewhere in the body except, with a much lower abundance, in the oral cavity.

20 Chemical skin permeation enhancers, for example dimethylsulfoxide, promote the diffusion of drugs across the skin by solubilising or extracting some of the intercellular lipids from the barrier. Transcutaneous transport is therefore most efficient in the least tightly packed lipid regions, where hydrophobic pores in the barrier are created most easily. Through such pores sufficiently small and lipophilic agents can diffuse along the transcutaneous concentration gradient(s). The resulting skin permeability is unaffected by the agent concentration, unless the agent acts as an enhancer, but the permeability depends on the concentration and the selection of skin permeation enhancer(s).

25 However the hydrophobic pores in the skin are not big enough to allow an appreciable transport of large drugs of any kind. Owing to the self-sealing

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tendency of the intercellular lipid domains the pores are also rather short lived. The lipophilicity of typical pores in the skin also precludes the transport of hydrophilic, that is, of highly polar, molecules across the organ. Conventional skin permeation enhancement is therefore only useful for the delivery of fatty materials which do not irritate the skin too much, the enhancer-mediated transport and irritation being poorly tolerated by the consumers in many cases.

Therefore to date, permeation based drug delivery through the skin is really successful only for small drugs with a molecular weight below 400 Da. Such drugs can partition into the intercellular lipid matrix in the skin and then diffuse through small hydrophobic pores in the horny layer, first into the skin proper and then further down towards the deep body tissues. The resulting steady state transport is preceded by a short lag-time period, during which the drug traverses the barrier. Transcutaneous transport does not suffer from the first pass effect, however.

The bioavailability of drugs delivered through the skin by such conventional means is typically below 50 %, and often does not even reach 25 % (Hadgraft, 1996; Cevc, 1997).

Large hydrophobic molecules normally cross the skin in negligible quantity only. As already mentioned above this is due to the lack of suitable passages in the skin. Transcutaneous transport of macromolecules therefore chiefly relies on the molecular diffusion through shunts, such as pilosebaceous units. To deliver a bulky and highly polar agent across the skin other methods than those conventionally used are therefore required. For example various skin poration techniques were introduced to create hydrophilic pores in the skin suitable for the purpose (to avoid confusion we will call such hydrophilic pores channels):

5 The simplest, and crudest solution, for making a wide channel through the skin is to eliminate mechanically the skin barrier. For example, to deliver a large, hydrophilic antidiuretic peptide 1-deamino-8-D-arginine vasopressin across the human skin from an occlusive patch the removal of a small piece of epidermis by vacu-suction has been used (Svedman et al., 1996).

10 Further, a most common method for opening a wide channel through the skin is to use an injection needle or mechanical impact(s) (injection; powderjection). Locally restricted skin challenge is also possible. This can be done by local heat application (thermoporation); by using high voltage pulses (>150 V; electroporation); or by acoustic energy, such as ultrasound (few W cm^{-2} ; sonoporation). The resulting channel size depends on the nature and intensity of the skin treatment, but not on the nature or the applied amount of molecules to be transported.

15 Openings or even craters in the skin created by the above mentioned methods heal rather slowly under normal application conditions; the wider the passage, the more so. The skin thus may behave as an adaptable, but slowly recoverable barrier.

20 Even the most commonly used methods for making pores in the skin rely on gadgets plus experience for the proper operation; they also involve skin disinfection to protect the patient. This notwithstanding, their harm and inconvenience is tolerated as long as therapeutic benefit is achieved.

25 The most recent tool for creating hydrophilic passages in those barriers, such as the skin is provided by microscopic barrier penetrants which directly and reversibly open said hydrophilic channels. Such penetrants are independent of external energy source and also do not rely on any gadgets. They are also well tolerated by the skin.

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Such penetrants known to date all belong to the class of highly deformable complex droplets (Transfersomes®). Such droplets adapt to the pores of the barrier - which they then cross efficiently - provided that the droplet components and preparation are properly selected and/or optimised. A sufficiently adaptable and hydrophilic droplet can therefore cross the barrier, such as skin, spontaneously. Such hydrophilic channels are opened transiently by the moving penetrant after the latter has adjusted its shape to achieve the goal. This allows the adjustable droplets to act as vehicles for the delivery of various - hydrophilic or hydrophobic - agents across the barrier.

Most useful droplets comprise an aqueous core surrounded by an highly flexible mixed lipid bilayer, which makes the aggregate ultradeformable and superficially highly hydrophilic. Both is required for an efficient transcutaneous transport (Cevc, 1997). Said droplets were demonstrated to transport their mass rather efficiently across the skin under optimum application conditions (Cevc, 1997).

Other types of aggregates (liposomes, niosomes, nanoparticles, microemulsions, etc.) also have been claimed to traverse the skin efficiently but were seldom, if ever, proven really to deliver the associated drugs across the skin in practically meaningful quantities. It is believed that in contrast to the highly deformable droplets (Transfersomes®) the used aggregates are either insufficiently deformable and/or are too unstable to achieve the goal. Conventional aggregates instead act as simple drug reservoirs on the skin: the aggregates, incapable of crossing the barrier, remain on the skin while the drug is released gradually from the 'vehicle' to then probably diffuse through the skin barrier on its own. The main action of conventional drug loaded suspensions is thus to increase the skin barrier hydration and/or to shed the molecules with the skin permeation enhancing capability into the tissue.

Contrary, the composite, ultradeformable lipid droplets (Transfersomes®) deform and then penetrate the skin rather than to coalesce locally. Such aggregates motion across the skin seems to proceed along the natural moisture gradient(s) between the skin cells, which guides the aggregates into the hydrophilic (virtual) channels in the organ.

The predecessors of those channels that let highly adaptable droplets pass through the skin are originally so narrow that they only permit evaporation of (rather small) water molecules across the skin. These originally tiny pores (diameter < 0.5 nm) seem to open reversibly, however, when the stress of partial dehydration of a droplet, which is thereby being forced into the channel mouth under non-occlusive conditions, becomes excessive. The strong hydrophilicity and the large mass of the droplet are the factors which maximise the droplets' tendency to move through the skin; however the droplet adaptability is the necessary condition for the success of said motion.

The movement of the droplets across the skin seems to proceed along the path pursued by the water molecules during the skin passage in the opposite direction. The droplets are thus guided into intercellular regions precisely at the points where the contacts between the above-cited skin sealing lipids are the weakest and the least tight. The corresponding skin region covered with the channels has been estimated to be around 4 % of the total skin area, or less.

It is possible to associate small and large, hydrophobic and hydrophilic molecules with ultradeformable and highly adaptable droplet-like aggregates. Using such complex aggregate droplets all types of molecules can thus be delivered across the barrier, such as the stratum corneum.

High systemic availabilities of the drug transported are typically achieved. Relative efficiency of the transport across the skin exceeds 50 %, in most cases (Cevc et al., 1996). The steady state is reached within few hours, by and large (Cevc et al., 1998).

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It has already been proven that the skin barrier recovers fully after those droplets have been eliminated from the skin surface. In contrast, the channels created by other means, such as ultrasound remain open for at least 20 hours. In fact, they are not resealed properly before 2 days, even when relatively weak therapeutic
10 ultrasound is used. Stronger perturbation causes more persistent skin damage (Mitragotri et al., 1995). (In the extreme case, when the barrier is eliminated by vacu-suction, the skin does not recover fully until after of 8 weeks.)

15

The precise size distribution of the channels in the skin, through which highly deformable droplets migrate spontaneously across the stratum corneum, is as yet unknown. It is probable, however, that it is asymmetric. The average width, that is, the distribution maximum has been estimated to be 20-30 nm under typically used application conditions. The skewed distribution could result from the existence of two quantitatively different but qualitatively similar intercellular
20 transport routes across the skin (Schätzlein & Cevc, 1998) which together form the family of transcutaneous pathways.

25

The first, inter-cluster pathway leads between the groups of corneocytes. It represents the high-end tail of channel-size distribution and typically starts at the bottom of inter-cluster gorges. From here, it follows the dense material filling such gorge and offers the lowest resistance to penetration at the junctions where several clusters meet.

The second, intra-cluster pathway leads between the individual corneocytes in each cluster of corneocytes. This route typically proceeds along the lipid layers surface. In the projection over the outer third of the stratum corneum, the inter-corneocyte pathway resembles an interwoven three-dimensional network including all the cells in the organ. (Schätzlein & Cevc, 1998).

The above mentioned distinctions are quantitative in nature. No doubt exists that transcutaneous channels with the exception of pilosebaceous units are resistant to the passage of non-deformable, large aggregates.

Channel properties are also sufficiently constant to reveal little inter-site, inter-individual, inter-species or inter-carrier variability. According to the prior art, the relative bio-availability of different drugs in the blood after an epicutaneous administration in highly adaptable droplets (Transfersomes®) is fairly constant (Cevc, 1997). Pore distribution depends little on the nature of the penetrant or the drug. The same has been implied for the dose dependence, which was concluded to affect merely the depth of penetrant and drug distribution. Small dose per area was found to favour the local (superficial) retention whereas a large dose per area was shown to ensure a relatively great systemic availability.

Surprisingly, and contrary to the above-mentioned conclusion, we have now found out that changing the applied dose above a certain threshold and in sufficiently wide range not only affects the drug/penetrant distribution, but also determines the rate of penetrant transport across the barrier.

Our new and unexpected finding provides means for controlling the rate of transcutaneous drug delivery whenever highly deformable carriers are used on the barrier; it also provides the basis for better, i.e. more rational, design of the delivery device. There will especially be profit for the development of cutaneous

patches suitable for the use in combination with highly adaptable carriers (Transfersomes®). Improved therapy and higher commercial value of the products should be the consequence.

5 It stands to reason that the observed new effect reflects the widening of channels in the barrier, but the applicant does not wish to be bound to this hypothesis. The newly found dosage-dependent pore widening is probably different for various transcutaneous channels: the originally narrower pores probably change more than the relatively wide (e.g. inter-cluster) channels. The effect of relative channel
10 size, that is, of channel vs. penetrant size ratio, suggests that it will take much longer time to bring certain penetrants quantity through narrow than through wide channels.

If the channels act as transported mass discriminators, and adjust their width to the
15 flux requirement, the narrow channels will persist much longer in their original, high penetration resistance state than the wide channels. However, after having responded to the multi-penetrant passage by increasing their width such channels will start to behave as the originally wider channels. Multiple adjustments are possible but only to certain upper limit.

20 Another potentially important factor acting in the same direction is the skin surface hydration, which is prone to increase with enlargement of the topically administered dosage. In either case, the average width and the size distribution of channels in the skin will shift towards greater values with increasing applied
25 dosage. This then will result in higher final transcutaneous flux.

For the avoidance of doubt, all pertinent information, definitions and lists from the previous patent applications of the same applicant are incorporated herein by reference.

Kits and more particularly devices for administering drugs through a barrier such as skin or mucosa have also already been described. These devices can typically be divided into matrix systems and liquid reservoir systems.

5

Container-type reservoirs are often formed as a pocket between the backing layer and a rate controlling membrane through which the drug passes to the skin. The pressure sensitive adhesive layer normally underlies the membrane and the drug also passes through it on its way to the skin.

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As mentioned above it is customary to prepare reservoir type patches for transdermal drug delivery with a backing membrane and a rate controlling membrane (Ogiso, T., Y. Ito, et al. (1989). "Membrane-controlled transdermal therapeutic system containing clonazepam and anticonvulsant activity after its application." *Chem Pharm Bull (Tokyo)* 37, 446-9; Ito, Y., T. Ogiso, et al. (1993). "Percutaneous absorption of acemetacin from a membrane controlled transdermal system and prediction of the disposition of the drug in rats" *Biol. Pharm. Bull* 16, 583-8)

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A number of reservoir type systems have been described.

US-Patent No. 829,224 to Chang et al., for instance, discloses a device with a reservoir that is defined by a backing layer and a drug-permeable membrane layer.

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A ring-shaped layer made of an adhesive is peripheral to the reservoir. A peelable liner layer underlies the membrane. A second peelable layer, the release liner, underlies the entire assembly. A first heat seal connects the backing layer and the membrane and surrounds the reservoir. A second heat seal concentric about the first heat seal connects the backing layer and the release liner. The second heat seal is broken when the release liner is removed. The device may include an inner

liner that underlies the membrane and portions of the backing layer. This inner liner is removed following removal of the release liner so that the membrane is exposed.

5 U.S.-Patent Nr. 4,983,395 to Chang et al., relates to another device with a backing layer and a membrane layer that define a reservoir. A peelable inner liner underlies the reservoir and portions of the backing and membrane layers outside the periphery of the reservoir. An adhesive layer underlies the inner liner and remaining portions of the backing and membrane layers. A peelable release liner
10 underlies the adhesive layer. A first heat seal connects the backing and membrane layers on the periphery of the reservoir. A second heat seal underlies the first heat seal and connects the membrane and the inner liner. In use, the release liner and inner liner are peeled away to expose the undersurfaces of the membrane and adhesive layers prior to placement of the device onto the skin or mucosa.

15 PCT-Application W096-19205 to Theratech, Inc., discloses a device for administering an active agent to the skin or mucosa of an individual comprising a laminated composite of an adhesive overlay, a backing layer underlying the central portion of the adhesive overlay, an active agent-permeable membrane, the backing
20 layer and membrane defining a reservoir that contains a formulation of the active agent, a peel seal disc underlying the active agent-permeable membrane, a heat seal about the periphery of the peel seal disc, the active agent-permeable membrane and the backing layer and a removable release liner underlying the exposed overlay and peel seal disc. The adhesive layer is above and peripheral to
25 the path of the active agent to the skin or mucosa and is protected from degradation by the components of the reservoir by a multiplicity of heat seals. The peel seal disc protects against release of the active agent-containing reservoir and the release liner protects the adhesive from exposure to the environment prior to use.

US-Patent No. 5,202,125 to Theratech, Inc., describes a transdermal delivery system for delivery of nitroglycerin which deliver the drug at enhanced transdermal fluxes. The systems include, in addition to nitroglycerin, a permeation enhancer which is either a sorbitan ester, a C8-C22 aliphatic alcohol, or a mixture thereof. Methods for administering nitroglycerin using such permeation enhancers are also disclosed.

WO90-11065 to Theratech, Inc., discloses a transdermal drug delivery device comprising a drug formulation containing reservoir defined by a backing layer and a drug-permeable membrane layer, a peelable inner liner that underlies the reservoir and a portion of the backing/membrane outwardly of the reservoir periphery, an adhesive layer that underlies the inner liner and outwardly extending portions of the membrane/backing layers, and a peelable release liner layer that underlies the adhesive layer with a first permanent heat seal between the backing and the membrane about the perimeter of the reservoir and another peelable (impermeant) heat seal between the membrane and the inner liner underlying the first permanent heat seal, the heat seals and peelable barrier layer providing barriers that isolate the drug formulation from the adhesive.

Depending on the features to be achieved, backing films are either occlusive or permeable and commonly are derived from synthetic polymers, such as polyester, polyethylene, polyvinylidene chloride (PVDC), polyurethane or natural polymers, such as cotton, wool, etc. It is possible to use nonporous, microporous, such as polypropylene or polyethylene or also macroporous woven and nonwoven materials as a backing layer in transdermal patches. The backing layers are generally selected from these materials depending on the active agent to be delivered.

Occlusive backings in classical TTS (transdermal transport systems) tend to promote higher deposition and a higher rate of permeation of the active or inactive ingredients into the skin compared to non-occlusive backing. Occlusive backings are e.g. desirable to enhance the delivery of steroids to the lower layers of the epidermis to treat inflammation and dermatoses. Examples are Actiderm®
5 (dermatological patch) or Cordran® (tape and patch).

Semi-occlusive films, such as polyurethanes and polyolefin copolymers, and non-occlusive woven and nonwoven fiber-based materials, such as cotton and polyester, allow water vapor transmission from the skin surface and from the patch. These semi-occlusive or non-occlusive materials are rarely used as backing materials in TTS. Thicker non-occlusive backings were only desirable for corn and callus removal products since the active agent needs only to be delivered to the outer layers of the stratum corneum. The non-occlusive woven and nonwoven materials used in many of these products mainly serve as a protective cushion.
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Rate controlling membranes usually used in commercial TTS are thin (26 – 78 µm) nonporous ethylene vinyl acetate films, such as Transderm-Nitro® (Ciba-Geigy and ZAFFARONI) Duragesic®, Estraderm®, and Estraderm®. Moreover, thin (26 – 78 µm) microporous films of polyethylene, such as Transderm-Scop®, Catapres® are used as rate controlling membrane in multilaminate solid state reservoir patches or in liquid reservoir TTS. Further examples for such microporous PE-membranes are β-Estro® and Androderm®. These membranes usually serve to limit the rate of diffusion of the drug onto and through the skin.
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As already described above Transfersomes® are able to mediate agent or drug delivery through the skin due to the hydration gradient across the biological barrier. In contrary to customary transdermal transport systems, wherein the agent mediation commonly depends on classical Fick's law of diffusion, therapeutic

systems suitable for Transfersomes® and useful for the method of the present invention must fulfill different criteria.

5 It is also problematic that Transfersome®-mediated drug delivery through the skin from a patch is hindered if an occlusive backing material is used. The use of an occlusive membrane as backing layer causes an increased Transfersomes® hydration, since e.g. vapors cannot leak from the patch. Accordingly the hydration gradient and therefore the driving force for the Transfersome® transport is dramatically lowered.

10 Another problem is that many of the non-occlusive woven and nonwoven backings, which customary serve as a protective cushion, retain the Transfersomes® due to adsorption and trapping of lipids and proteins in the fibrous structure.

15 Moreover, any classical microporous and non-porous rate-controlling membranes having a pore size of smaller than about 20 nm may interfere with the passage of Transfersomes® through the pores due to size exclusion.

20 It is obvious to someone skilled in the art that the known transdermal patches having conventional backing and rate controlling membranes are not suitable for the mediation of Transfersomes® according to the present invention. The same applies to matrix-type patches.

25 In matrix-type transdermal patches are those in which the drug is contained in and released from a polymer matrix. The matrix is typically made of a pressure sensitive adhesive and defines the basal surface of the patch (i.e. the surface affixed to the skin).

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A number of matrix type systems have been described.

US-Patent No. 5,460,820 to Theratech, Inc., discloses a method of providing testosterone replacement therapy to a woman in need of such therapy comprising
5 applying a testosterone-delivering patch to the skin of said woman which patch transdermally delivers 50 to 500 µg/day testosterone to the woman. The skin patch comprises a laminated composite of a backing layer and a matrix layer comprising a solution of testosterone in a polymeric carrier, said matrix layer providing a sufficient daily dose of testosterone to provide said therapy.

10 US-Patent No. 5,783,208 to Theratech, Inc., discloses a matrix-type transdermal patch for coadministering estradiol and another steroid wherein the matrix is composed of a N-vinyl-2-pyrrolidone-containing acrylic copolymer pressure sensitive adhesive, estradiol the other steroid, and optionally a permeation
15 enhancer, and the respective fluxes of estradiol and the other steroid from the matrix are independent of the respective concentrations of the other steroid and estradiol in the matrix.

20 All pertinent information, definitions and lists from the patents and patent applications of the US-company Theratech, Inc. are expressively incorporated herein by reference.

25 As mentioned above, it is customary to prepare reservoir type patches for transdermal drug delivery with a backing membrane and a rate controlling membrane. These membranes form typically one compartment, which contains the corresponding formulation. This can be a - mostly alcoholic or aqueous - solution, an aqueous suspension or a gel which contains gel forming polymers. Parameters as chemical and physical stability, viscosity, concentrations of active ingredient(s) and excipients are not critical with respect to commercial one-compartment

reservoir-types, since the currently most active ingredients (drugs) are stable, low-molecular-weight substances (nicotine, fentanyl, estradiol, scopolamin and others), which commonly do not interfere with e.g. additional ingredients such as antioxidants, stabilizers, cosolvents or penetration enhancers.

5

As already mentioned, the Transfersome®-mediated drug delivery through barriers clearly differs from customary drug delivery through the skin. While it is not possible administering high molecular drugs by transdermal patches known in the art, Transfersomes® in principle are suitable carriers for a drug of high molecular weight such as peptides (e.g. insulin) and proteins (serum albumin). It is clear to someone skilled in the art that problems may arise if e.g. labile proteins are mixed with interfering or destabilizing ingredients over an extended storage period in customary one-compartment patches.

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In many cases sufficient stabilities of all ingredients are not achievable within one compartment. For example Transfersome®-forming phospholipids are most stable at pH 6.5, while proteins may have other pH values of optimal stability (e.g. Interferon- α -2b at pH = 7.4 or pH = 3). Therefore, it would be necessary to keep said substances in different media if stored over an extended time period. For example, Transfersomes of type-T are formulated and stable in phosphate-buffer, while hepatocyte growth factor (HGF) is stable in citrate-buffer. Moreover, commonly organic (co-)solvents are used to introduce antioxidants such as BHT into lipid aggregates. Said (co-)solvents may contribute to reduced solubility of the proteins as they lower the bulk dielectricity constant, thus reducing electrostatic repulsion. This may lead to uncontrolled, at least unwanted, aggregation and denaturation of the proteins.

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It is an important object of the present invention to control the flux of highly deformable penetrants (Transfersomes®) across an adaptable semi-permeable

porous barrier, such as the skin of a human or animal body or a plant. It is another object of the present invention to control the flux of highly deformable penetrants (Transfersomes®) across an adaptable semi-permeable porous barrier in using a kit or transdermal transport system which enables the formulation to be applied at the selected dose per area. It is a further object of the present invention to provide a reservoir-type transdermal patch suitable for the Transfersome®-mediated agent or drug delivery through the intact skin. Another object of the present invention is the provision of a long term stable multicompartment reservoir-type transdermal patch, which comprises separate compartments and is suitable for the Transfersome®-mediated agent or drug delivery through the intact skin.

According to the present invention this is achieved by a method for controlling the flux of penetrants across an adaptable semi-permeable porous barrier comprising the steps of:

- 15 – preparing a formulation by suspending or dispersing said penetrants in a polar liquid in the form of fluid droplets surrounded by a membrane-like coating of one or several layers, said coating comprising at least two kinds or forms of amphiphilic substances with a tendency to aggregate, provided that
- 20 – said at least two substances differ by at least a factor of 10 in solubility in said polar liquid,
- and / or said substances when in the form of homo-aggregates (for the more soluble substance) or of hetero-aggregates (for any combination of both said substances) have a preferred average diameter smaller than the diameter of homo-aggregates containing merely the less soluble substance,
- 25 – and / or the more soluble substance tends to solubilise the droplet and the content of such substance is to up to 99 mol-% of solubilising concentration or else corresponds to up to 99 mol-% of the saturating concentration in the unsolubilised droplet, whichever is higher;

- and /or the presence of the more soluble substance lowers the average elastic energy of the membrane-like coating to a value at least 5 times lower, more preferably at least 10 times lower and most preferably more than 10 times lower, than the average elastic energy of red blood cells or of phospholipid bilayers with fluid aliphatic chains,
- said penetrants being able to transport agents through the pores of said barrier or to enable agent permeation through the pores of said barrier after penetrants have entered the pores,
- selecting a dose amount of said penetrants to be applied on a predetermined area of said barrier to control the flux of said penetrants across said barrier, and
- applying the selected dose amount of said formulation containing said penetrants onto said area of said porous barrier.

Preferably the flux of penetrants across said barrier is increased by enlarging the applied dose amount of said penetrants.

It then is preferred if the pH of the formulation is between 3 and 10, more preferably is between 4 and 9, and most preferably is between 5 and 8.

According to another preferred feature of the present invention the formulation containing the penetrants comprises:

- at least one thickening agent in an amount to increase the formulation viscosity to maximally 5 Nm/s, more preferably up to 1 Nm/s, and most preferably up to 0.2 Nm/s, so that formulation spreading-over, and drug retention at the application area is enabled,
- and / or at least one antioxidant in an amount that reduces the increase of oxidation index to less than 100 % per 6 months, more preferably to less than 100 % per 12 months and most preferably to less than 50 % per 12 months

- and / or at least one microbicide in an amount that reduces the bacterial count of 1 million germs added per g of total mass of the formulation to less than 100 in the case of aerobic bacteria, to less than 10 in the case of entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa* or *Staphylococcus aureus*, after a period of 4 days.

It then is preferred if said at least one microbicide is added in an amount that reduces the bacterial count of 1 million germs added per g of total mass of the formulation to less than 100 in the case of aerobic bacteria, to less than 10 in the case of entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa* or *Staphylococcus aureus*, after a period of 3 days, and more preferably after a period of 1 day.

It then is also preferred if said thickening agent is selected from the class of pharmaceutically acceptable hydrophilic polymers, such as partially etherified cellulose derivatives, like carboxymethyl-, hydroxyethyl-, hydroxypropyl-, hydroxypropylmethyl- or methyl-cellulose; completely synthetic hydrophilic polymers such as polyacrylates, polymethacrylates, poly(hydroxyethyl)-, poly(hydroxypropyl)-, poly(hydroxypropylmethyl)methacrylates, polyacrylonitriles, methallyl-sulphonates, polyethylenes, polyoxiethylenes, polyethylene glycols, polyethylene glycol-lactides, polyethylene glycol-diacrylates, polyvinylpyrrolidones, polyvinyl alcohols, poly(propylmethacrylamides), poly(propylene fumarate-co-ethylene glycols), poloxamers, polyaspartamides, (hydrazine cross-linked) hyaluronic acids, silicones; natural gums comprising alginates, carrageenans, guar-gums, gelatines, tragacanth, (amidated) pectins, xanthans, chitosan collagens, agaroses; mixtures and further derivatives or co-polymers thereof and / or other pharmaceutically, or at least biologically, acceptable polymers.

Preferrably the concentration of said polymer is chosen to be in the range between 0.01 w- % and 10 w- %, more preferably in the range between 0.1 w- % and 5 w- %, even more preferably in the range between 0.25 w- % and 3.5 w- % and most preferably in the range between 0.5 w- % and 2 w- %.

5

Further it is preferred that said anti-oxidant is selected from synthetic phenolic antioxidants, such as butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT) and di-tert-butylphenol (LY178002, LY256548, HWA-131, BF-389, CI-986, PD-127443, E-5119, BI-L-239XX, etc.), tertiary butylhydroquinone (TBHQ),
10 propyl gallate (PG), 1-O-hexyl-2,3,5-trimethylhydroquinone (HTHQ); aromatic amines (such as diphenylamine, p-alkylthio-o-anisidine, ethylenediamine derivatives, carbazol, tetrahydroindenoindol); phenols and phenolic acids (such as guaiacol, hydroquinone, vanillin, gallic acids and their esters, protocatechuic acid, quinic acid, syringic acid, ellagic acid, salicylic acid, nordihydroguaiaretic acid
15 (NDGA), eugenol); tocopherols (including tocopherols (alpha, beta, gamma, delta) and their derivatives, such as tocopheryl-acylate (e.g. -acetate, -laurate, myristate, -palmitate, -oleate, -linoleate, etc., or any other suitable tocopheryl-lipoate), tocopheryl-POE-succinate; trolox and corresponding amide- and thiocarboxamide analogues; ascorbic acid and its salts, isoascorbate, (2 or 3 or 6)-o-alkylascorbic
20 acids, ascorbyl esters (e.g. 6-o-lauroyl, myristoyl, palmitoyl-, oleoyl, or linoleoyl-L-ascorbic acid, etc.); non-steroidal anti-inflammatory agents (NSAIDs), such as indomethacin, diclofenac, mefenamic acid, flufenamic acid, phenylbutazone, oxyphenbutazone acetylsalicylic acid, naproxen, diflunisal, ibuprofen, ketoprofen, piroxicam, penicillamine, penicillamine disulphide,
25 primaquine, quinacrine, chloroquine, hydroxychloroquine, azathioprine, phenobarbital, acetaminophen); aminosalicic acids and derivatives; methotrexate, probucol, antiarrhythmics (e.g. amiodarone, aprindine, asocainol), ambroxol, tamoxifen, b-hydroxytamoxifen; calcium antagonists (such as nifedipine, nisoldipine, nimodipine, nicardipine, nilvadipine), beta-receptor

blockers (e.g. atenolol, propranolol, nebivolol); sodium bisulphite, sodium metabisulphite, thiourea; chelating agents, such as EDTA, GDTA, desferal; endogenous defence systems, such as transferrin, lactoferrin, ferritin, ceruloplasmin, haptoglobin, haemopexin, albumin, glucose, ubiquinol-10; enzymatic antioxidants, such as superoxide dismutase and metal complexes with a similar activity, including catalase, glutathione peroxidase, and less complex molecules, such as beta-carotene, bilirubin, uric acid; flavonoids (e.g. flavones, flavonols, flavonones, flavanones, chalcones, anthocyanins), N-acetylcysteine, mesna, glutathione, thiohistidine derivatives, triazoles; tannins, cinnamic acid, hydroxycinnamic acids and their esters (e.g. coumaric acids and esters, caffeic acid and their esters, ferulic acid, (iso-) chlorogenic acid, sinapic acid); spice extracts (e.g. from clove, cinnamon, sage, rosemary, mace, oregano, allspice, nutmeg); carnosic acid, carnosol, carsoic acid; rosmarinic acid, rosmarinol, gentisic acid, ferulic acid; oat flour extracts, such as avenanthramide 1 and 2; thioethers, dithioethers, sulfoxides, tetraalkylthiuram disulphides; phytic acid, steroid derivatives (e.g. U74006F); tryptophan metabolites (e.g. 3-hydroxykynurenine, 3-hydroxyanthranilic acid), and organochalcogenides, or else is an oxidation suppressing enzyme.

Then, the concentration of BHA or BHT is often chosen to be between 0.001 and 2 w-%, more preferably is between 0.0025 and 0.2 w-%, and most preferably is between 0.005 and 0.02 w-%, of TBHQ and PG is between 0.001 and 2 w-%, more preferably is between 0.005 and 0.2 w-%, and most preferably is between 0.01 and 0.02 w-%, of tocopherols is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.075 w-%, of ascorbic acid esters is between 0.001 and 5, more preferably is between 0.005 and 0.5, and most preferably is between 0.01 and 0.15 w-%, of ascorbic acid is between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01 and 0.1 w-%, of sodium bisulphite or sodium

metabisulphite is between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01-0.15 w-%, of thiourea is between 0.0001 and 2 w-%, more preferably is between 0.0005 and 0.2, and most preferably is between 0.001-0.01 w-%, most typically 0.005 w-%, of cystein is
5 between 0.01 and 5, more preferably is between 0.05 and 2 w-%, and most preferably is between 0.1 and 1.0 w-%, most typically 0.5 w-%, of monothioglycerol is between 0.01 and 5 w-%, more preferably is between 0.05 and 2 w-%, and most preferably is between 0.1-1.0 w-%, most typically 0.5 w-%, of NDGA is between 0.0005-2 w-%, more preferably is between 0.001-0.2 w-%,
10 and most preferably is between 0.005-0.02 w-%, most typically 0.01 w-%, of glutathione is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.2 w-%, most typically 0.1 w-%, of EDTA is between 0.001 and 5 w-%, even more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01 and 0.2 w-%, most
15 typically between 0.05 and 0.975 w-%, of citric acid is between 0.001 and 5 w-%, even more preferably is between 0.005 and 3 w-%, and most preferably is between 0.01-0.2, most typically between 0.3 and 2 w-%.

Furthermore it is preferred if said microbicide is selected amongst short chain
20 alcohols, such as ethyl and isopropyl alcohol, chlorbutanol, benzyl alcohol, chlorbenzyl alcohol, dichlorbenzylalcohol; hexachlorophene; phenolic compounds, such as cresol, 4-chloro-m-cresol, p-chloro-m-xlenol, dichlorophene, hexachlorophene, povidon-iodine; parabens, especially alkyl-paraben, such as methyl-, ethyl-, propyl-, or butyl-paraben, benzyl-paraben;
25 acids, such as sorbic acid, benzoic acid and its salts; quaternary ammonium compounds, such as alkonium salts, e.g. benzalkonium salts, especially the chlorides or bromides, cetrimonium salts, e.g. the bromide; phenoalkecinium salt, such as phenododecinium bromide, cetylpyridinium chloride or other such salts; mercurium compounds, such as phenylmercuric acetate, borate, or nitrate,

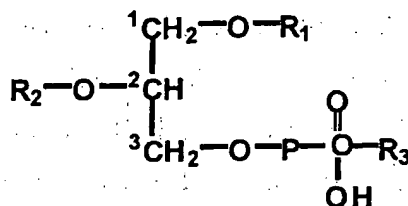
thiomersal; chlorhexidine or its gluconate; antibiotically active compounds of biological origin, or a mixture thereof.

Preferrably the bulk concentration of short chain alcohols in the case of ethyl, propyl, butyl or benzyl alcohol is up to 10 w-%, more preferably is up to 5 w-%, and most preferably is in the range between 0.5-3 w-%, and in the case of chlorobutanol is in the range between 0.3-0.6 w-%; bulk concentration of parabens, especially in the case of methyl paraben is in the range between 0.05-0.2 w-%, and in the case of propyl paraben is in the range between 0.002-0.02 w-%; bulk concentration of sorbic acid is in the range between 0.05-0.2 w-%, and in the case of benzoic acid is in the range between 0.1-0.5 w-%; bulk concentration of phenols, triclosan, is in the range between 0.1-0.3 w-%, and bulk concentration of chlorhexidine is in the range between 0.01-0.05 w-%.

It is preferred that the less soluble amongst the aggregating substances is a lipid or lipid-like material, especially a polar lipid, whereas the substance which is more soluble in the suspending liquid and which lowers the average elastic energy of the droplet is a surfactant or else has surfactant-like properties and / or is a form of said lipid or lipid-like material which is comparably soluble as said surfactant or the surfactant-like material.

Preferrably the lipid or lipid-like material is a lipid or a lipoid from a biological source or a corresponding synthetic lipid or any of its modifications, said lipid preferably belonging to the class of pure phospholipids corresponding to the general formula

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where R₁ and R₂ is an aliphatic chain, typically a C₁₀₋₂₀-acyl, or -alkyl or partly unsaturated fatty acid residue, in particular, an oleoyl-, palmitoeloyl-, elaidoyl-,
 5 linoleyl-, linolenyl-, linolenoyl-, arachidoyl-, vaccinyl-, lauroyl-, myristoyl-, palmitoyl-, or stearoyl chain; and where R₃ is hydrogen, 2-trimethylamino-1-ethyl, 2-amino-1-ethyl, C₁₋₄-alkyl, C₁₋₅-alkyl substituted with carboxy, C₂₋₅-alkyl substituted with hydroxy, C₂₋₅-alkyl substituted with carboxy and hydroxy, or C₂₋₅-alkyl substituted with carboxy and amino, inositol, sphingosine, or salts of said
 10 substances, said lipid comprising also glycerides, isoprenoid lipids, steroids, sterines or sterols, of sulphur- or carbohydrate-containing lipids, or any other bilayer-forming lipids, in particular half-protonated fluid fatty acids, said lipid is selected from the group comprising phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositols,
 15 phosphatidic acids, phosphatidylserines, sphingomyelins or other sphingophospholipids, glycosphingolipids (including cerebrosides, ceramidepolyhexosides, sulphatides, sphingoplasmalogens), gangliosides and other glycolipids or synthetic lipids, in particular with corresponding sphingosine derivatives, or any other glycolipids, whereby two similar or different chains can
 20 be ester-groups-linked to the backbone (as in diacyl and dialkenoyl compound) or be attached to the backbone with ether bonds, as in dialkyl-lipids.

The surfactant or surfactant-like material preferably is a nonionic, a zwitterionic, an anionic or a cationic surfactant, especially a fatty-acid or -alcohol, an alkyl-

tri/di/methyl-ammonium salt, an alkylsulphate salt, a monovalent salt of cholate, deoxycholate, glycocholate, glycodeoxycholate, taurodeoxycholate, taurocholate, etc., an acyl- or alkanoyl-dimethyl- aminoxide, esp. a dodecyl- dimethyl- aminoxide, an alkyl- or alkanoyl-N-methylglucamide, N- alkyl-N,N-
 5 dimethylglycine, 3-(acyldimethylammonio)-alkanesulphonate, N-acyl- sulphobetaine, a polyethylene-glycol-octylphenyl ether, esp. a nonaethylene-glycol-octylphenyl ether, a polyethylene-acyl ether, esp. a nonaethylen-dodecyl ether, a polyethylene-glycol-isoacyl ether, esp. a octaethylene-glycol-isotridecyl ether, polyethylene-acyl ether, esp. octaethylenedodecyl ether, polyethylene-
 10 glycol-sorbitane-acyl ester, such as polyethylenglykol-20-monolaurate (Tween 20) or polyethylenglykol-20-sorbitan-monooleate (Tween 80), a polyhydroxyethylene-acyl ether, esp. polyhydroxyethylene- lauryl, -myristoyl, -cetylstearyl, or -oleoyl ether, as in polyhydroxyethylene-4 or 6 or 8 or 10 or 12, etc., -lauryl ether (as in Brij series), or in the corresponding ester, e.g. of polyhydroxyethylen-8-stearate
 15 (Myrj 45), -laurate or -oleate type, or in polyethoxylated castor oil 40, a sorbitane-monoalkylate (e.g. in Arlacel or Span), esp. sorbitane-monolaurate, an acyl- or alkanoyl-N-methylglucamide, esp. in or decanoyl- or dodecanoyl-N-methylglucamide, an alkyl-sulphate (salt), e.g. in lauryl- or oleoyl-sulphate, sodium deoxycholate, sodium glycodeoxycholate, sodium oleate, sodium taurate,
 20 a fatty acid salt, such as sodium elaidate, sodium linoleate, sodium laurate, a lysophospholipid, such as n-octadecylene(=oleoyl)-glycerophosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-acyl-, e.g. lauryl or oleoyl-glycero-phosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-tetradecyl-glycero-phosphatidic acid, -phosphorylglycerol, or - phosphorylserine, a
 25 corresponding palmitoeloyl-, elaidoyl-, vaccenyl-lysophospholipid or a corresponding short-chain phospholipid, or else a surface-active polypeptide.

According to a preferred feature of the present invention, the average diameter of the penetrant is between 30 nm and 500 nm, more preferably between 40 nm and

250 nm, even more preferably between 50 nm and 200 nm and particularly preferably between 60 nm and 150 nm.

5 It is another preferred feature of the present invention that the total dry weight of droplets in a formulation is 0.01 weight-% (w-%) to 40 w-% of total formulation mass, more preferably is between 0.1 w-% and 30 w-%, and most preferably is between 0,5 w-% and 20 w-%.

10 It is preferred that the total dry weight of droplets in a formulation is selected to increase the formulation viscosity to maximally 200 mPas, more preferably up to 40 mPas, and most preferably up to 8 mPas.

15 According to the present invention is is preferred if at least one edge-active substance or surfactant and/or at least one amphiphilic substance, and / or at least one hydrophilic fluid and the agent are mixed, if required separately, to form a solution, the resulting (partial) mixtures or solutions are then combined subsequently to induce, preferably by action of mechanical energy such as shaking, stirring, vibrations, homogenisation, ultrasonication, shearing, freezing and thawing, or filtration using convenient driving pressure, the formation of
20 penetrants that associate with and / or incorporate the agent

25 Preferrably this amphiphilic substances are dissolved in volatile solvents, such as alcohols, especially ethanol, or in other pharmaceutically acceptable organic solvents, such as ethanol, 1- and 2-propanol, benzyl alcohol, propylene glycol, polyethylene glycol (molecular weight: 200-400 D) or glycerol, other pharmaceutically acceptable organic solvents, such as undercooled gas, especially supercritical CO₂, which are then removed, especially by evaporation or dilution, prior to making the final preparation.

According to the present invention the formation of said penetrants preferably is induced by the addition of required substances into a fluid phase, evaporation from a reverse phase, by injection or dialysis, if necessary under the influence of mechanical stress, such as shaking, stirring, especially high velocity stirring, vibrating, homogenising, ultrasonication, shearing, freezing and thawing, or filtration using convenient, especially low (1 MPa) or intermediate (up to 10 MPa), driving pressure.

Then the formation of said penetrants preferably is induced by filtration, the filtering material having pores sizes between 0.01 μm and 0.8 μm , more preferably between 0.02 μm and 0.3 μm , and most preferably between 0.05 μm and 0.15 μm , whereby several filters may be used sequentially or in parallel.

According to the invention said agents and penetrants preferably are made to associate, at least partly,

- after the formation of said penetrants, e.g. after injecting a solution of the drug in a pharmaceutically acceptable fluid, such as ethanol, 1- and 2-propanol, benzyl alcohol, propylene glycol, polyethylene glycol (molecular weight: 200-400 D) or glycerol into the suspending medium,
- simultaneously with penetrant formation, if required using the drug co-solution and, at least some, penetrant ingredients.

It is preferred if said penetrants, with which the agent is associated are prepared immediately before the application of the formulation, if convenient, from a suitable concentrate or a lyophilisate.

The formulation according to the invention preferably is applied by spraying, smearing, rolling or sponging on the application area, in particular by using a metering sprayer, spender, roller, sponge or a non-occlusive patch, as appropriate.

It is preferred if the barrier is a part of a mammalian body and / or a plant and preferably is skin and / or at least partly keratinised endothelium and / or nasal or any other mucosa.

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The area dose of said penetrant then preferably is between 0.1 mg per square centimetre (mg cm^{-2}) and 40 mg cm^{-2} , more preferably is between 0.25 mg cm^{-2} and 30 mg cm^{-2} and even more preferably is between 0.5 mg cm^{-2} and 15 mg cm^{-2} , in the case that the penetrant is applied on said skin and / or said at least partly

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keratinised endothelium.

The area dose of said penetrant then preferably is between 0.0001 mg per square centimetre (mg cm^{-2}) and 0.1 mg cm^{-2} , more preferably is between 0.0005 mg cm^{-2} and 0.05 mg cm^{-2} and even more preferably is between 0.001 mg cm^{-2} and 0.01

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mg cm^{-2} , in the case that the penetrant is applied on plant body, plant leaves or plant needles.

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The area dose of said penetrant then preferably is between 0.05 mg per square centimetre (mg cm^{-2}) and 20 mg cm^{-2} , more preferably is between 0.1 mg cm^{-2} and 15 mg cm^{-2} and even more preferably is between 0.5 mg cm^{-2} and 10 mg cm^{-2} , in the case that the penetrant is applied on said nasal or other mucosa.

25

In another advantageous aspect of the invention, a kit containing said formulation in an amount which enables the formulation to be applied at the selected dose per area as afore-mentioned is provided.

It then is preferred if the formulation is contained in a bottle or any other packaging vessel.

The kit preferably contains a device for administering the formulation.

According to another aspect of the present invention a patch is provided containing the formulation in an amount that yields the dose per area as mentioned
5 above. The patch or transdermal patch according to the present invention is intended for the application to barriers including the skin, mucosa or plants. The term "transdermal" should include these aforesaid barriers.

Preferably the patch comprises

- 10
- a non-occlusive backing liner;
 - an inner liner, wherein the backing liner and the inner liner define a reservoir; and /or a matrix layer.

It is preferred that said non-occlusive backing liner exhibits a mean vapor
15 transmission rate (MVTR) of more than 1000 g/m²day, preferably of more than 5.000 g/m²day and most preferably of more than 10.000 g/m²day. It is preferred that the non-occlusive backing liner has pores of smaller than 100 nm, preferably smaller than 70 nm, more preferably of smaller than 30 nm and most preferably as big as the inter-molecular distances of the backing material. In a further preferred
20 embodiment the non-occlusive backing liner comprises a polyurethane membrane, preferably a polyester track-etched porous membrane, more preferably a polycarbonate track-etched porous membrane and most preferably a polyethylene microporous membrane.

25 The inner liner and / or matrix layer according to the present invention establishes skin contact. The inner liner preferably prevents unwanted release of the formulation from the patch during storage and enables rapid skin wetting when contacted with the skin. According to the present invention it is further preferred that the inner liner comprises a homogeneous membrane, preferably a polyester

track-etched porous membrane or a polycarbonate track-etched porous membrane. Moreover, these inner liner membranes preferably have a pore density of up to 5%, preferably of up to 15%, more preferably of up to 25% and most preferably of more than 25% and/or a pore size in the range between 20 nm and 200 nm, preferably between 50 nm and 140 nm and most preferably between 80 nm and 120 nm.

Further preferred inner liner materials comprise a hydrophobic mesh-membrane and/or a nonwoven fleece with mesh openings formed by hydrophobic threads. In another preferred embodiment the inner liner is a microporous polyethylene membrane having average pore sizes in the range of between 50 nm to 3000 nm, preferably between 500 nm to 2000 nm and most preferably of about 1500 nm.

According to a further preferred embodiment of the present invention the patch comprises a pressure sensitive adhesive layer, preferably an adhesive layer comprising polyacrylate, polyisobutylene, silicone, ethylene vinyl acetate copolymer, polyvinylpyrrolidone or polyethylene oxide hydrogel.

According to another preferred feature of the present invention the formulation comprises penetrants having an average diameter of smaller than 150 nm, preferably of smaller than 100 nm. It is also preferred that the total dry weight of droplets in the formulation is at least 5 weight-% (w-%), preferably between 7.5 w-% and 30 w-%, and more preferably between 10 w-% and 20 w-%.

The patch according to the present invention preferably comprises a formulation, wherein the formulation up to maximally 200 mPas, more preferably up to 40 mPas, and most preferably up to 8 mPas.

The area of the drug releasing membrane is between 0.5 cm^2 and 250 cm^2 , more preferably is between 1 cm^2 and 100 cm^2 , even more preferably is between 2 cm^2 and 50 cm^2 and most preferred is between 4 cm^2 and 25 cm^2 .

- 5 In an especially preferred embodiment it is preferred that the patch comprises one or more additional layers comprising desiccant containing layers, matrix layers, foam tape layers and/or protective layers.

- 10 The inventors found that it is advantageous to use backing liners having the capability to support the evaporation of the Transfersomes suspending medium. According to the present invention they preferably exhibit a mean vapor transmission rate (MVTR) of more than $1000 \text{ g/m}^2\text{day}$ or, better, more than $10000 \text{ g/m}^2\text{day}$. The solvent disappearance across such a barrier at sufficiently high rate helps to create and to maintain an activity gradient which drives the flux of
- 15 Transfersome®-aggregates across a barrier.

- Suited inventive backing liners are polyurethane membranes, such as CoTran 9701 (3M Medica, Borken Germany), Tegaderm (3M Medica, Borken Germany), Arcare 8311 (Adhesive Research, Limerick, Ireland), IV3000 (Smith and
- 20 Nephew). Even better suited are polyester track-etched porous membranes (10 nm pore size) (Osmonics, Minnetonka, USA) and polycarbonate track-etched porous membranes (10 nm pore size) (Osmonics, Minnetonka, USA). Most suited are the polyethylene microporous membranes such as Cotran 9711 (3M Medica, Borken Germany), 14P01A, 10P05A, 8P07A, E011 D (DSM Solutech, Heerlen, The
- 25 Netherlands). In classical TTS known in the art, the latter materials customary are used for rate controlling membranes.

Said backing liner need to be liquid-tight in order to prevent loss of active substance, which should be delivered e.g. transdermally. In order to ensure or

determine if the membrane is liquid-tight, the penetrability of Transfersomes® through the membranes is measured upon application of low hydrostatic pressures. The polyethylene membranes Cotran 9711 (3M Medica, Borken Germany) and 14P01A are liquid tight up to an applied pressure of 1 MPa. Further, all cited
5 polyurethane membranes are liquid tight.

Another important feature of the patch according to the present invention is the use of an inner liner membrane instead of conventional rate controlling membranes which enable rapid skin wetting with the Transfersome®-formulation, while blocking the (unwanted) release of the formulation during storage or during
10 the application of the device on the skin. Since the present invention specifically is directed to Transfersome®-containing patches, the term "rate controlling membrane" is misleading, since the rate of Transfersome® mediated transport is ideally controlled by the water activity in and on the biological barrier. Thus, the
15 term "inner liner" is used herein instead of "rate controlling membrane".

One inner liner membrane, which is suitable for the purpose of the present invention is a homogeneous membrane having a high pore density. The passage through the pores depends on the Laplace pressure / surface tension of lipid
20 suspension within the pores $P_{\min} = 2 \sigma \cos \theta / r$, where P_{\min} denotes the minimal pressure required to overcome the Laplace pressure, σ is the surface tension of the suspension-air interface (~ 30 mN/m), θ is the contact angle of the formulation on the membrane material and r is the pore radius (~ 100 nm). Accordingly, retention of the formulation in the pores requires $\cos \theta < 0$, which means that the membrane
25 needs to be hydrophobic. According to this possible theory a Laplace pressure of 0.6 MPa is needed to move the air-suspension interface through the pores, thus enabling the suspension to cross the barrier.

Well suited inner liner membrane materials according to the present invention are polyester track-etched porous membranes (100 nm pore size) (Infiltec, Speyer, Germany) and polycarbonate track-etched porous membranes (100 nm pore size) (Infiltec, Speyer, Germany).

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Moreover, it is intended by the inventors to use hydrophobic mesh-membranes e.g. Fluortex 09/70/22, Fluortex 09/85/27 (INFILTEC, Speyer) and nonwoven fleeces e.g. Parafil R20, Parafil RK 20, Parafil R 30 Natur, Parafil RK 30, Paratherm PR 220/18, Paratherm PR 220/20 (LTS, Andernach, Germany). These sieving materials are well suited to act as inner liner in inventive patches.

10

Said liners constitute mesh openings built up by the hydrophobic threads. They prevent the passage of Transfersomes® when the liner is not in contact with the skin. The high contact angle γ of the air/water or air/Transfersome®-suspension interface, with respect to the hydrophobic surface of the thread, ensures this. The mesh openings allow for the passage of the Transfersomes® through the liner when contacting the skin. This is caused by the energy gained by the wetting of a more hydrophilic or of a less hydrophobic surface (e.g. the skin) exceeding the surface energy needed for the complete wetting of the threads.

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In more concrete terms, said "switching-effect" can be explained as follows: Let d be the distance between two threads from midpoint to midpoint. Let r be the radius of a thread:

25

$$2 \pi r z \gamma_{wt} \approx z d \gamma_{ws}$$

The surface tension of water on the skin is $\gamma_{ws} = 40 \text{ m N/m}$ according to "Transdermal and Drug Delivery Systems", Buffalo Grove, Interpharm Press, Ghosh, Pfister et al. 1997. The surface tension of water on the hydrophobic thread

is $\gamma_{wt} = 70 \text{ m N/m}$. (The surface tension of a suspension on skin is again $\gamma_{ws} = 40 \text{ m N/m}$, the surface tension of the suspension on the hydrophobic thread is $\gamma_{wt} = 35 \text{ m N/m}$, due to the presence of a detergent monolayer). Rearranging the above formula yields

5

$$2 \pi r / d \approx \gamma_{ws} / \gamma_{wt}$$

for the case of the suspension ($\gamma_{ws} \sim \gamma_{wt}$). This suggests that the thread radius to mesh size ratio should preferably be in the range of about 0.3.

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According to the present invention it is especially preferred to use microporous polyethylene membranes as inner liner. The term "microporous" for the purposes of the present invention means pore sizes of at least 20 nm, preferably in the range between 50 nm to 3000 nm. Examples are Solupor - E011 D (mean pore size 1500

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nm), Solupor - 8P07A (mean pore size 700 nm) and Solupor - 10P05A (mean pore size 500 nm) (DSM Solutech, Heerlen, The Netherlands), which exhibit a high penetrability at small pressures thus allowing for Transfersomes to wet the skin upon contact.

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For all types of the above mentioned inner liner membranes the surface tension, σ , and the contact angle, γ , are changed when contacted with the skin. There are various factors, which can cause said changes of the surface tension, σ , and the contact angle, γ . One factor may be an increase in humidity and capillary condensation of transepidermally released water. Hydrophilic bridging due to

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interaction between corneocytes / hair follicles and the inner membrane may also contribute to rapid skin wetting. Finally, hydrophilisation of the pore core by contaminants, such as microscopic skin fragments, may alter the surface tension, σ , and the contact angle, γ . As a consequence, the minimal pressure P_{\min} , which is

required to overcome the Laplace pressure, is reduced and the formulation can pass the inner liner and wet the skin surface.

5 Patches according to the present invention can be manufactured by different methods known in the art. On principle the lamination of the backing and the inner liner can be carried out by heat lamination or adhesive lamination or any other known lamination method.

10 In heat lamination processes the liners are adhered by melting at least one material at elevated temperatures and elevated pressures for short periods. The melt(s) merge and intercalate upon cooling and consolidation. The temperature and pressure is applied by metallic chops, either pulse heated, e.g. by microwave radiation, or continuously heated. Polyethylene and polyurethan membranes typically are heat laminated at temperatures of 120 - 200 °C, preferably of 140 –
15 160 °C and pressures of 1-6 bar, preferably of 3-4 bar. Good lamination properties are achieved for Tranfersom® containing patches by applying a pressure of 4 bar for a period of about 0.1 - 5 seconds, preferably of about 1-2 seconds.

20 Adhesive lamination of the liners is achieved by a layer of pressure sensitive adhesive such as polyacrylate, polyisobutylene, silicone, ethylene vinyl acetate copolymer or polyvinylpyrrolidone and polyethylene oxide hydrogel adhesive (PVP/PEO). The adhesive liner is precut to the appropriate shape for example a concentric ring having a width of 1 cm. The backing and the inner liners are laminated to the ring and the patch is punched out of the web. Suitable films are
25 for example a pressure sensitive transfer film (Arcare 7396), a flexible plastic film coated on both sides with a medical grade pressure sensitive adhesive (Arcare 8570 clear polyester) or foam tapes (Polyolefin 3M 1777; 3M 1779; 3M 9751, polyvinyl chloride 3M 9772L) coated on both sides with pressure sensitive acrylate adhesive. The latter example mounts a reservoir of defined volume due to

the finite thickness of the foam tape, while the former two examples draw their Transfersome® containing volume by the elasticity and/or hidden area of the liners.

- 5 The filling of the one compartment reservoir type patch according to the present invention can be achieved by several methods known in the art.

One possible filling procedure is based on a two-step lamination process. In the first step, the main compartment is laminated while retaining a small orifice.
10 Through this port a tap or a tubing is induced and the Transfersome® formulation is injected into the preformed reservoir. After retraction of the tap or tubing the lamination of the port is finalized. Heat lamination as well as adhesive lamination can be used in said procedure. In the case of heat lamination the heat chop laminates a C-shaped ring. After the filling of the inner part of the C, the heat
15 chop is revolved by 45° and the heat lamination is repeated a second time now closing the open part of the C. In the case of adhesive lamination the release liner of the transfer tape is not removed completely thus allowing for the establishment of the filling port. After filling the rest of the release liner is removed and the port is sealed. Back-folding of the backing and/or the inner liner leads to the same
20 result: A collar-like port is formed, which is sealed by refolding the membranes after the filling process.

The form, fill and seal technique is well established and can also be used for the manufacture of the patches according to the present invention. In a first step the
25 film for the backing liner is moved over a trough of desired dimensions. The liner adopts this shape under vacuum and lines the trough. Then a tap fills the Transfersome® formulation into the trough. After the tap is retracted the inner liner membrane is applied onto the web. A concentric seal ring laminates both films either by heat lamination or adhesive lamination as described above.

In a further suitable process for making TTS the Transfersome® formulation is injected through a preinstalled tubing after the lamination process. The tubing is laterally inserted into the foam in the same way as a venous catheter is set for continuous injection. The tubing is connected to a Transfersome®-formulation filled syringe by a luer lock. The desired amount of formulation is injected into the reservoir and the tubing is removed and /or sealed if necessary.

In another important aspect of the present invention a patch is provided which is further characterised in that the patch comprises at least two compartments, which are separated from each other during storage. According to another aspect of the present invention a patch is provided containing the formulation in an amount that yields the dose per area as mentioned above, wherein the patch comprises several, more preferably less than 5, even more preferably 3, and most preferred 2 separate inner compartments which are combined prior to or during the application of the formulation. Preferably at least one of the compartments is inside and /or outside the patch.

It is preferred that the formulation and / or the individual formulation components and/or the agent and / or the suspension / dispersion of penetrants without the agent are kept during the storage in several, preferably less than 5, more preferably in 3, and most preferred in 2 separate compartments of the patch which, in case, are combined prior to or during or after the application of the patch.

In another preferred embodiment the outer compartments comprise injection systems, preferably syringes, which are connected to the reservoir of the patch. It is preferred that the compartments are vertically stacked and /or are arranged side-by-side and / or one compartment is included in a second compartment, preferably without being fixed to the second compartment.

5 Preferably the compartments are inside the reservoir, which is defined by the backing liner and the inner liner. It is further preferred that the compartments are separated from each other by a controllably openable barrier, preferably a membrane and /or by a plug and / or by a compartment-forming lamination.

10 According to the present invention combining and mixing of the ingredients of the compartments is achieved by direct mechanical action, such as pressing, rubbing, kneading, twisting, tearing and /or indirectly by changing the temperature, osmotic pressure or electrical potential, thereby causing the removal or destroying of the separating barrier(s).

15 In a further preferred embodiment of the present invention the patch comprises
— an inventive non-occlusive backing liner
— a membrane defining a reservoir, which is divided in at least two compartments,
wherein the formulation directly contacts the skin when the formulation releases from the reservoir or compartments.

20 The inventive multicompartment reservoir-type patch comprises at least two separate compartments and a mixing compartment, wherein said mixing compartment may be an storage compartment containing one ingredient of the formulation or the formulation or may be an compartment, which is not filled
25 during the storage period.

According to the present invention the storage compartments containing the critical ingredients may be separated from the mixing compartment. The storage compartments are containing some, if not all, ingredients during the storage period

after preparation and prior to application. The mixing compartment serves to mix the separated ingredients after the storage period. After mixing the formulation is released onto the skin from the mixing compartment. The mixing compartment may have an adjustable area of skin contact to allow for area-dose control. This
5 can be done by the merger of smaller subunits of mixing compartments.

The mixing compartment has to be in contact with the skin. This can be achieved either by

1. direct contact with the skin (no inner liner membrane) or
- 10 2. an inner liner membrane according to the present invention. Reference is made to the one-compartment patch described above. The identical inner liner membranes may be used for multicompartment TTS.

The number of storage compartments may be at least two and is depending on the
15 respective longterm-incompatibilities of the ingredients.

The storage compartments may be part of the patch and may be made of the same material(s). The storage compartments may be - in the simplest form - two
20 syringes containing the liquid ingredients, which are injected sequentially or simultaneously into the mixing chamber through one or more tubes. A twin-syringe of which the two pistons are connected facilitates simultaneous injection and constancy of the ingredients ratio. An additional tubing ideally with micro-arcs as used in HPLC sample preparation may cause turbulences of the merged
25 liquid. A T-piece connector, ideally with turbulence chamber serves in the same manner. Thus, an optimal mixing of the components is achieved even at high viscosities and high lipid-concentrations.

The mixing compartment according to the present invention may be one separate compartment which is empty during storage but filled almost simultaneously,

when the patch is applied onto the skin, or it may be one of the existing storage compartments in which the other ingredients are being added from other storage compartments, or it may be created by the merger of two or more storage compartments.

5

The combining or mixing of the ingredients can be achieved by perforating or destroying the compartment-separating membranes. This can be done, for example, by pressing or kneading the patch such that the compartment-separating membranes rupture upon this mechanical stress, or by the external or internal
10 activation of a sharp tool, such as a needle by perforating the compartment-separating membrane.

15

Another method combining or mixing of the ingredients is based on opening a tube-system between the compartments. Said opening can be achieved e.g. by pressing or kneading the patch such that plug or squid which close the tubing between the separated compartments during the storage-period is released from the tubing due to the applied pressure.

20

It is also possible according to present invention to combine and mix the ingredients by unsealing of a lamination, which forms the separated storage compartments. This can be done, for example, by applying a small but a steady-state pressure onto the filled storage chambers, but also by heat lamination or adhesive lamination. The lamination of the compartment-forming membranes
25 unseals and the liquids squeeze through the self-formed channels into the mixing compartment.

The storage and mixing compartments may be stacked vertically or placed side-by-side. For example, three membranes can be laminated in a manner that half of the middle membrane is sealed to the lower (e.g. inner liner) membrane and the

other half is sealed to the upper membrane (backing liner). Upper and lower membranes are sealed at the edges on the very right, very left, forward-turned and backward-turned sides thus forming a two-compartment pouch. The middle membrane might be impermeable to liquids, but also easy to disrupt. Suitable materials for middle membranes might be e.g. thin polyurethanes. According to one possible embodiment the storage container for the Transfersomes®-formulation may be the left liquid-tight compartment, while the Transfersome®-release is performed from the right chamber through the inventive inner liner membrane when contacted to the skin. The right chamber may serve e.g. as a storage compartment for (lyophilized) drug(s). It is clear to someone skilled in the art that also combinations of the aforementioned embodiments, e.g. a combination of the vertical stacking and side-by-side alignment are suitable for the purposes of the present invention.

After the mixing process in the mixing compartment the emptied storage compartments are dispensable. They may be unplugged (in the case of external compartments, such as syringes) or clipped off. For example the tubes may be detached and the ports may be sealed with tape or squids or plugs. Open sealing may be re-laminated by applying pressure.

It another important aspect of the present invention, a method is provided of administering an agent to a mammalian body or a plant, by transporting said agent through a barrier, wherein the barrier is the intact skin, mucosa and/or cuticle of said mammalian body or a plant, said agent being associated to a penetrant capable of transporting said agent through the skin pores or through the passages in mucosa or cuticle, or capable of enabling agent permeation through skin pores after said penetrant has opened and/or entered said pores, comprising the steps of:

- preparing a formulation by suspending or dispersing said penetrants in a polar liquid in the form of fluid droplets surrounded by a membrane-like coating of

one or several layers, said coating comprising at least two kinds or forms of amphiphilic substances with a tendency to aggregate, provided that

— said at least two substances differ by at least a factor of 10 in solubility in said polar liquid,

5 — and / or said substances when in the form of homo-aggregates (for the more soluble substance) or of hetero-aggregates (for any combination of both said substances) have a preferred average diameter smaller than the diameter of homo-aggregates containing merely the less soluble substance,

10 — and / or the more soluble substance tends to solubilise the droplet and the content of such substance is to up to 99 mol-% of solubilising concentration or else corresponds to up to 99 mol-% of the saturating concentration in the unsolubilised droplet, whichever is higher,

15 — and / or the presence of the more soluble substance lowers the average elastic energy of the membrane-like coating to a value at least 5 times lower, more preferably at least 10 times lower and most preferably more than 10 times lower, than the average elastic energy of red blood cells or of phospholipid bilayers with fluid aliphatic chains,

20 — said penetrants being able to transport agents through the pores of said barrier or being able to promote agent permeation through the pores of said skin after penetrants have entered the pores,

— selecting a dose amount of said penetrants to be applied on a predetermined area of said barrier to control the flux of said penetrants across said barrier, and

25 — applying the selected dose amount of said formulation containing said penetrants onto said area of said porous barrier.

It then is preferred if the flux across said barrier is increased by enlarging the applied dose amount of said penetrants per area of barrier.

The pH of the formulation preferably is chosen to be between 3 and 10, more

preferably is between 4 and 9, and most preferably is between 5 and 8.

In this aspect of the invention, it then is preferred if the formulation comprises:

- 5 – at least one thickening agent in an amount to increase the formulation viscosity to maximally 5 Nm/s, more preferably up to 1 Nm/s, and most preferably up to 0.2 Nm/s, so that formulation spreading-over, and drug retention at the application area is enabled,
- 10 – and / or at least one antioxidant in an amount that reduces the increase of oxidation index to less than 100 % per 6 months, more preferably to less than 100 % per 12 months and most preferably to less than 50 % per 12 months
- 15 – and / or at least one microbicide in an amount that reduces the bacterial count of 1 million germs added per g of total mass of the formulation to less than 100 in the case of aerobic bacteria, to less than 10 in the case of entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa* or *Staphilococcus aureus*, after a period of 4 days.

20 Said at least one microbicide then preferably is added in an amount that reduces the bacterial count of 1 million germs added per g of total mass of the formulation to less than 100 in the case of aerobic bacteria, to less than 10 in the case of entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa* or *Staphilococcus aureus*, after a period of 3 days, and more preferably after a period of 1 day.

25 Said thickening agent preferably is selected from the class of pharmaceutically acceptable hydrophilic polymers, such as partially etherified cellulose derivatives, like carboxymethyl-, hydroxyethyl-, hydroxypropyl-, hydroxypropylmethyl- or methyl-cellulose; completely synthetic hydrophilic polymers such as polyacrylates, polymethacrylates, poly(hydroxyethyl)-, poly(hydroxypropyl)-, poly(hydroxypropylmethyl)methacrylates, polyacrylonitriles, methallyl-

5 sulphonates, polyethylenes, polyoxiethylenes, polyethylene glycols, polyethylene glycol-lactides, polyethylene glycol-diacrylates, polyvinylpyrrolidones, polyvinyl alcohols, poly(propylmethacrylamides), poly(propylene fumarate-co-ethylene glycols), poloxamers, polyaspartamides, (hydrazine cross-linked) hyaluronic acids, silicones; natural gums comprising alginates, carrageenans, guar-gums, gelatines, tragacanth, (amidated) pectins, xanthans, chitosan collagens, agaroses; mixtures and further derivatives or co-polymers thereof and / or other pharmaceutically, or at least biologically, acceptable polymers.

10 The concentration of said polymer then preferably is chosen to be in the range between 0.01 w- % and 10 w- %, more preferably in the range between 0.1 w- % and 5 w- %, even more preferably in the range between 0.25 w- % and 3.5 w- % and most preferably in the range between 0.5 w- % and 2 w- %.

15 According to the invention said anti-oxidant then preferably is selected from synthetic phenolic antioxidants, such as butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT) and di-tert-butylphenol (LY178002, LY256548, HWA-131, BF-389, CI-986, PD-127443, E-5119, BI-L-239XX, etc.), tertiary butylhydroquinone (TBHQ), propyl gallate (PG), 1-O-hexyl-2,3,5-trimethylhydroquinone (HTHQ); aromatic amines (such as diphenylamine, 20 p-alkylthio-o-anisidine, ethylenediamine derivatives, carbazol, tetrahydroindenoindol); phenols and phenolic acids (such as guaiacol, hydroquinone, vanillin, gallic acids and their esters, protocatechuic acid, quinic acid, syringic acid, ellagic acid, salicylic acid, nordihydroguaiaretic acid (NDGA), 25 eugenol); tocopherols (including tocopherols (alpha, beta, gamma, delta) and their derivatives, such as tocopheryl-acylate (e.g. -acetate, -laurate, myristate, -palmitate, -oleate, -linoleate, etc., or any other suitable tocopheryl-lipoate), tocopheryl-POE-succinate; trolox and corresponding amide- and thiocarboxamide analogues; ascorbic acid and its salts, isoascorbate, (2 or 3 or 6)-o-alkylascorbic

acids, ascorbyl esters (e.g. 6-o-lauroyl, myristoyl, palmitoyl-, oleoyl, or linoleoyl-L-ascorbic acid, etc.); non-steroidal anti-inflammatory agents (NSAIDs), such as indomethacin, diclofenac, mefenamic acid, flufenamic acid, phenylbutazone, oxyphenbutazone acetylsalicylic acid, naproxen, diflunisal, ibuprofen, ketoprofen, piroxicam, penicillamine, penicillamine disulphide, 5 primaquine, quinacrine, chloroquine, hydroxychloroquine, azathioprine, phenobarbital, acetaminophen); aminosalicylic acids and derivatives; methotrexate, probucol, antiarrhythmics (e.g. amiodarone, aprindine, asocainol), ambroxol, tamoxifen, b-hydroxytamoxifen; calcium antagonists (such as 10 nifedipine, nisoldipine, nimodipine, nicardipine, nilvadipine), beta-receptor blockers (e.g. atenolol, propranolol, nebivolol); sodium bisulphite, sodium metabisulphite, thiourea; chelating agents, such as EDTA, GDTA, desferal; endogenous defence systems, such as transferrin, lactoferrin, ferritin, ceruloplasmin, haptoglobin, haemopexin, albumin, glucose, ubiquinol-10; 15 enzymatic antioxidants, such as superoxide dismutase and metal complexes with a similar activity; including catalase, glutathione peroxidase, and less complex molecules, such as beta-carotene, bilirubin, uric acid; flavonoids (e.g. flavones, flavonols, flavonones, flavanones, chalcones, anthocyanins), N-acetylcysteine, mesna, glutathione, thiohistidine derivatives, triazoles; tannins, cinnamic acid, 20 hydroxycinnamic acids and their esters (e.g. coumaric acids and esters, caffeic acid and their esters, ferulic acid, (iso-) chlorogenic acid, sinapic acid); spice extracts (e.g. from clove, cinnamon, sage, rosemary, mace, oregano, allspice, nutmeg); carnosic acid, carnosol, carsolic acid; rosmarinic acid, rosmarindiphenol, gentisic acid, ferulic acid; oat flour extracts, such as avenanthramide 1 and 2; 25 thioethers, dithioethers, sulfoxides, tetraalkylthiuram disulphides; phytic acid, steroid derivatives (e.g. U74006F); tryptophan metabolites (e.g. 3-hydroxykynurenine, 3-hydroxyanthranilic acid), and organochalcogenides, or else is an oxidation suppressing enzyme.

It then is preferred if the concentration of BHA or BHT is between 0.001 and 2 w-%, more preferably is between 0.0025 and 0.2 w-%, and most preferably is between 0.005 and 0.02 w-%, of TBHQ and PG is between 0.001 and 2 w-%, more preferably is between 0.005 and 0.2 w-%, and most preferably is between 0.01 and 0.02 w-%, of tocopherols is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.075 w-%, of ascorbic acid esters is between 0.001 and 5, more preferably is between 0.005 and 0.5, and most preferably is between 0.01 and 0.15 w-%, of ascorbic acid is between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01 and 0.1 w-%, of sodium bisulphite or sodium metabisulphite is between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01-0.15 w-%, of thiourea is between 0.0001 and 2 w-%, more preferably is between 0.0005 and 0.2, and most preferably is between 0.001-0.01 w-%, most typically 0.005 w-%, of cystein is between 0.01 and 5, more preferably is between 0.05 and 2 w-%, and most preferably is between 0.1 and 1.0 w-%, most typically 0.5 w-%, of monothioglycerol is between 0.01 and 5 w-%, more preferably is between 0.05 and 2 w-%, and most preferably is between 0.1-1.0 w-%, most typically 0.5 w-%, of NDGA is between 0.0005-2 w-%, more preferably is between 0.001-0.2 w-%, and most preferably is between 0.005-0.02 w-%, most typically 0.01 w-%, of glutathione is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.2 w-%, most typically 0.1 w-%, of EDTA is between 0.001 and 5 w-%, even more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01 and 0.2 w-%, most typically between 0.05 and 0.975 w-%, of citric acid is between 0.001 and 5 w-%, even more preferably is between 0.005 and 3 w-%, and most preferably is between 0.01-0.2, most typically between 0.3 and 2 w-%.

Preferrably said microbicide is then selected amongst short chain alcohols, such as ethyl and isopropyl alcohol, chlorbutanol, benzyl alcohol, chlorbenzyl alcohol, dichlorbenzylalcohol; hexachlorophene; phenolic compounds, such as cresol, 4-chloro-m-cresol, p-chloro-m-xlenol, dichlorophene, hexachlorophene, 5 povidon-iodine; parabens, especially alkyl-paraben, such as methyl-, ethyl-, propyl-, or butyl-paraben, benzyl-paraben; acids, such as sorbic acid, benzoic acid and its salts; quaternary ammonium compounds, such as alkonium salts, e.g. benzalkonium salts, especially the chlorides or bromides, cetrimonium salts, e.g. the bromide; phenoalkecinium salt, such as phenododecinium bromide, 10 cetylpyridinium chloride or other such salts; mercurium compounds, such as phenylmercuric acetate, borate, or nitrate, thiomersal; chlorhexidine or its gluconate; antibiotically active compounds of biological origin, or a mixture thereof.

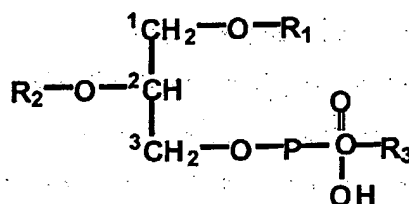
15 It then is preferred that the bulk concentration of short chain alcohols in the case of ethyl, propyl, butyl or benzyl alcohol is up to 10 w-%, more preferably is up to 5 w-%, and most preferably is in the range between 0.5-3 w-%, and in the case of chlorobutanol is in the range between 0.3-0.6 w-%; bulk concentration of parabens, especially in the case of methyl paraben is in the range between 20 0.05-0.2 w-%, and in the case of propyl paraben is in the range between 0.002-0.02 w-%; bulk concentration of sorbic acid is in the range between 0.05-0.2 w-%, and in the case of benzoic acid is in the range between 0.1-0.5 w-%; bulk concentration of phenols, triclosan, is in the range between 0.1-0.3 w-%, and bulk concentration of chlorhexidine is in the range between 0.01-0.05 w-%.

25

It then is also preferred that the less soluble amongst the aggregating substances is a lipid or lipid-like material, especially a polar lipid, whereas the substance which is more soluble in the suspending liquid and which lowers the average elastic energy of the droplet is a surfactant or else has surfactant-like properties and / or is

a form of said lipid or lipid-like material which is comparably soluble as said surfactant or the surfactant-like material.

5 Preferrably the lipid or lipid-like material is a lipid or a lipoid from a biological source or a corresponding synthetic lipid or any of its modifications, said lipid preferably belonging to the class of pure phospholipids corresponding to the general formula



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where R_1 and R_2 is an aliphatic chain, typically a C_{10-20} -acyl, or -alkyl or partly unsaturated fatty acid residue, in particular, an oleoyl-, palmitoeloyl-, elaidoyl-, linoleyl-, linolenyl-, linolenoyl-, arachidoyl-, vaccinyl-, lauroyl-, myristoyl-, palmitoyl-, or stearoyl chain; and where R_3 is hydrogen, 2-trimethylamino-1-ethyl, 2-amino-1-ethyl, C_{1-4} -alkyl, C_{1-5} -alkyl substituted with carboxy, C_{2-5} -alkyl substituted with hydroxy, C_{2-5} -alkyl substituted with carboxy and hydroxy, or C_{2-5} -alkyl substituted with carboxy and amino, inositol, sphingosine, or salts of said substances, said lipid comprising also glycerides, isoprenoid lipids, steroids, sterines or sterols, of sulphur- or carbohydrate-containing lipids, or any other bilayer-forming lipids, in particular half-protonated fluid fatty acids, said lipid is selected from the group comprising phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositols, phosphatidic acids, phosphatidylserines, sphingomyelins or other sphingophospholipids, glycosphingolipids (including cerebroside,

15

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ceramidopolyhexosides, sulphatides, sphingoplasmalogens), gangliosides and other glycolipids or synthetic lipids, in particular with corresponding sphingosine derivatives, or any other glycolipids, whereby two similar or different chains can be ester-groups-linked to the backbone (as in diacyl and dialkenoyl compound) or
5 be attached to the backbone with ether bonds, as in dialkyl-lipids.

The surfactant or surfactant-like material preferably is a nonionic, a zwitterionic, an anionic or a cationic surfactant, especially a fatty-acid or -alcohol, an alkyl-tri/di/methyl-ammonium salt, an alkylsulphate salt, a monovalent salt of cholate,
10 deoxycholate, glycocholate, glycodeoxycholate, taurodeoxycholate, taurocholate, etc., an acyl- or alkanoyl-dimethyl- aminoxide, esp. a dodecyl- dimethyl- aminoxide, an alkyl- or alkanoyl-N-methylglucamide, N- alkyl-N,N- dimethylglycine, 3-(acyldimethylammonio)-alkanesulphonate, N-acyl- sulphobetaine, a polyethylene-glycol-octylphenyl ether, esp. a nonaethylene-
15 glycol-octylphenyl ether, a polyethylene-acyl ether, esp. a nonaethylen-dodecyl ether, a polyethylene-glycol-isoacyl ether, esp. a octaethylene-glycol-isotridecyl ether, polyethylene-acyl ether, esp. octaethylenedodecyl ether, polyethylene- glycol-sorbitane-acyl ester, such as polyethylenglykol-20-monolaurate (Tween 20) or polyethylenglykol-20-sorbitan-monooleate (Tween 80), a polyhydroxyethylene-
20 acyl ether, esp. polyhydroxyethylene- lauryl, -myristoyl, -cetylstearyl, or -oleoyl ether, as in polyhydroxyethylene-4 or 6 or 8 or 10 or 12, etc., -lauryl ether (as in Brij series), or in the corresponding ester, e.g. of polyhydroxyethylen-8-stearate (Myrj 45), -laurate or -oleate type, or in polyethoxylated castor oil 40, a sorbitane- monoalkylate (e.g. in Arlacel or Span), esp. sorbitane-monolaurate, an acyl- or
25 alkanoyl-N-methylglucamide, esp. in or decanoyl- or dodecanoyl-N- methylglucamide, an alkyl-sulphate (salt), e.g. in lauryl- or oleoyl-sulphate, sodium deoxycholate, sodium glycodeoxycholate, sodium oleate, sodium taurate, a fatty acid salt, such as sodium elaidate, sodium linoleate, sodium laurate, a lysophospholipid, such as n-octadecylene(=oleoyl)-glycerophosphatidic acid, -

phosphorylglycerol, or -phosphorylserine, n-acyl-, e.g. lauryl or oleoyl-glycero-phosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-tetradecyl-glycero-phosphatidic acid, -phosphorylglycerol, or -phosphorylserine, a corresponding palmitoeloyl-, elaidoyl-, vaccenyl-lysophospholipid or a
5 corresponding short-chain phospholipid, or else a surface-active polypeptide.

The average diameter of the penetrant preferably is between 30 nm and 500 nm, more preferably between 40 nm and 250 nm, even more preferably between 50 nm and 200 nm and particularly preferably between 60 nm and 150 nm.

10

The total dry weight of droplets in a formulation is then preferably chosen to range from 0.01 weight-% (w-%) to 40 w-% of total formulation mass, more preferably is between 0.1 w-% and 30 w-%, and most preferably is between 0,5 w-% and 20 w-%.

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Preferably the total dry weight of droplets in a formulation is selected to increase the formulation viscosity to maximally 200 mPas, more preferably up to 40 mPas, and most preferably up to 8 mPas.

20

Preferably at least one edge-active substance or surfactant and/or at least one amphiphilic substance, and / or at least one hydrophilic fluid and the agent are mixed, if required separately, to form a solution, the resulting (partial) mixtures or solutions are then combined subsequently to induce, preferably by action of mechanical energy such as shaking, stirring, vibrations, homogenisation,
25 ultrasonication, shearing, freezing and thawing, or filtration using convenient driving pressure, the formation of penetrants that associate with and / or incorporate the agent

It also is preferred if said amphiphilic substances then are dissolved in volatile solvents, such as alcohols, especially ethanol, or in other pharmaceutically acceptable organic solvents, such as ethanol, 1- and 2-propanol, benzyl alcohol, propylene glycol, polyethylene glycol (molecular weight: 200-400 D) or glycerol, other pharmaceutically acceptable organic solvents, such as undercooled gas, especially supercritical CO₂, which are then removed, especially by evaporation or dilution, prior to making the final preparation.

The formation of said penetrants then preferably is induced by the addition of required substances into a fluid phase, evaporation from a reverse phase, by injection or dialysis, if necessary under the influence of mechanical stress, such as shaking, stirring, especially high velocity stirring, vibrating, homogenising, ultrasonication, shearing, freezing and thawing, or filtration using a convenient, especially low (1 MPa) or intermediate (up to 10 MPa), driving pressure.

It then is also preferred if the formation of said penetrants is induced by filtration, the filtering material having pores sizes between 0.01 µm and 0.8 µm, more preferably between 0.02 µm and 0.3 µm, and most preferably between 0.05 µm and 0.15 µm, whereby several filters may be used sequentially or in parallel.

Said agents and penetrants are made to associate, at least partly,

- after the formation of said penetrants, e.g. after injecting a solution of the drug in a pharmaceutically acceptable fluid, such as ethanol, 1- and 2-propanol, benzyl alcohol, propylene glycol, polyethylene glycol (molecular weight: 200-400 D) or glycerol into the suspending medium,
- simultaneously with penetrant formation, if required using the drug co-solution and, at least some, penetrant ingredients.

It then is preferred if said penetrants, with which the agent is associated, are prepared immediately before the application of the formulation, if convenient, from a suitable concentrate or a lyophilisate.

- 5 Accordingly the formulation is applied by spraying, smearing, rolling or sponging on the application area, in particular by using a metered sprayer, spender, roller or a sponge, or a non-occlusive patch, as appropriate.

- 10 It further is preferred if the barrier is skin or at least partly keratinised endothelium and / or nasal or any other mucosa.

- 15 The area dose of said penetrant then preferably is between 0.1 mg per square centimetre (mg cm^{-2}) and 40 mg cm^{-2} , more preferably is between 0.25 mg cm^{-2} and 30 mg cm^{-2} and even more preferably is between 0.5 mg cm^{-2} and 15 mg cm^{-2} , in the case that the penetrant is applied on said skin and / or said at least partly keratinised endothelium.

- 20 The area dose of said penetrant preferably is between 0.05 mg per square centimetre (mg cm^{-2}) and 20 mg cm^{-2} , more preferably is between 0.1 mg cm^{-2} and 15 mg cm^{-2} and even more preferably is between 0.5 mg cm^{-2} and 10 mg cm^{-2} , in the case that the penetrant is applied on said nasal or other mucosa.

- 25 The area dose of said penetrant preferably is between 0.0001 mg per square centimetre (mg cm^{-2}) and 0.1 mg cm^{-2} , more preferably is between 0.0005 mg cm^{-2} and 0.05 mg cm^{-2} and even more preferably is between 0.001 mg cm^{-2} and 0.01 mg cm^{-2} , in the case that the penetrant is applied on plant body, plant leaves or plant needles.

It is preferred if the method is used for generating an immune response on a human or other mammal by vaccinating said mammal.

5 It is preferred if the method is used for generating a therapeutic effect in a human or other mammal.

According to the present invention the above mentioned method is preferably used for the treatment of inflammatory disease, dermatosis, kidney or liver failure, adrenal insufficiency, aspiration syndrome, Behcet syndrome, bites and stings, blood disorders, such as cold-haemagglutinin disease, haemolytic anemia, hypereosinophilia, hypoplastic anemia, macroglobulinaemia, trombocytopenic purpura, furthermore, for the management of bone disorders, cerebral oedema, Cogan's syndrome, congenital adrenal hyperplasia, connective tissue disorders, such as lichen, lupus erythematosus, polymyalgia rheumatica, polymyositis and dermatomyositis, epilepsy, eye disorders, such as cataracts, Graves' ophthalmopathy, haemangioma, herpes infections, neuropathies, retinal vasculitis, scleritis, for some gastro-intestinal disorders, such as inflammatory bowel disease, nausea and oesophageal damage, for hypercalcaemia, infections, e.g. of the eye (as in infections mononucleosis), for Kawasaki disease, myasthenia gravis, various pain syndromes, such as postherpetic neuralgia, for polyneuropathies, pancreatitis, in respiratory disorders, such as asthma, for the management of rheumatoid disease and osteoarthritis, rhinitis, sarcoidosis, skin diseases, such as alopecia, eczema, erythema multiforme, lichen, pemphigus and pemphigoid, psoriasis, pyoderma gangrenosum, urticaria, in case of thyroid and vascular disorders.

25

Without any limitation of the scope of the present invention as defined by the attached claims the invention shall now be described in more detail by referring to the following examples and figures only showing non-limiting embodiments of the present invention.

General experimental set-up and sample preparation

Test formulation. Highly adaptable aggregate droplets used within the framework of this work had the form of (oligo)bilayer vesicles. Typically, the test formulation contained biocompatible (phospho)lipids, such as phosphatidylcholine, and (bio)surfactants, such as sodium cholate or polysorbate (Tween 80). Different phospholipid/detergent ratios have been chosen to maintain or select the highest possible aggregate deformability.

Manufacturing was done as described in previous applications of the applicant. In short, a solution of phosphatidylcholine (SPC; Natterman Phospholipids, Cologne, Germany) in chloroform was labelled with the tritiated SPC (Amersham, XXX) and mixed with sodium cholate (Merck, Darmstadt, Germany) to obtain a phospholipid/detergent ratio of 3.75/1 (mol/mol). The mixture was dispersed in phosphate buffer (pH = 7.2) to yield a 10 w-% total lipid suspension.

Vesicles in the suspension were frozen and thawed three times. Subsequently, the formulation was passed under pressure through several micro-porous filters (first 200 nm; then 100 nm, and finally 50 nm or 80 nm; Poretics, CA). To check the reproducibility of vesicle manufacturing, the average size of vesicles was measured with dynamic light scattering procedure and found to be in the range of 80 nm to 150 nm.

Test animals. Mice of NMRI strain were 8 to 12 weeks old at the time of experimentation. They had free access to standard chow and water and were kept in suspension cages in groups of 4 to 6. Prior to test formulation administration, the application area on each animals back was shaved carefully. The test preparation was administered under general anaesthesia (0.3 mL per mouse of an isotonic NaCl solution containing 0.0071 % Rompun (Bayer, Leverkusen,

Germany) and 14.3 mg/mL Ketavet (Parke-Davis, Rochester, N.Y). The administration was done with a high precision pipette on the skin which was left non-occluded. Each animal was finally transferred into an individual cage where it was kept for a day. A different cage was used for each animal for at least 24 hrs.

5 4 animals were used per test group.

Test measurements. Blood samples were collected from tail end, after termination of experiment at least. In one set of experiments, the early blood sampling was done every 2 hrs. Organ samples included: liver, spleen, kidney, and skin. The latter was also inspected superficially, by taking 10 strips (using a Tesa-Film).

Processing the organ samples was done according to standard procedures: for ^3H -measurement, a small part of each organ and 100 μL of the carcass lysate were used to get the desired and quoted experimental data. These were analysed according to the standard procedures.

To determine total label recovery, the carcass of test animals was dissolved and discharged by addition of 50 mL perchloric acid

20

Recovery (% of applied activity) was determined and the recovered doses (% of applied activity per organ) as well as the total delivered amount [μg lipid/g organ] were calculated.

25

Examples 1-5:**Short term administration**

- 5 Highly adaptable complex droplets (ultradeformable vesicles; Transfersomes)
 87.4 mg phosphatidylcholine from soy bean (SPC)
 12.6 mg sodium cholate (NaChol)
 trace amount of ^3H -DPPC with specific activity: 750 $\mu\text{Ci}/500\mu\text{L}$
 0.9 mL phosphate buffer, pH 7.3
- 10 Duration of experiment: 8 h .
 Application area: 1 cm² on the upper dorsum. The various doses applied on the
 test area are given in the following table.

	Group 1	Group 2	Group 3	Group 4	Group 5
Applied volume [μL]	1.0	5.0	7.0	15.0	30.0
Appl. lipid amount [mg]	0.10	0.50	0.75	1.50	3.00
Applied activity [cpm]	108998	544991	817486	1634972	3269943

- 15 Results of test measurements are given in figures 1 to 6.

Examples 6-8:**Longer term administration**

20

Highly adaptable complex droplets (ultradeformable vesicles; Transfersomes)

- 87.4 mg phosphatidylcholine from soy bean (SPC)
 12.6 mg sodium cholate (NaChol)
 0.9 mL phosphate buffer, pH 7.3
 trace amount of ^3H -DPPC with specific activity: 250 $\mu\text{Ci}/\text{mL}$
- 25

Duration of experiment: 24 h.

Application area: 1 cm squared; dose per area is given in the following table.

	Group 6	Group 7	Group 8
Applied volume [μ L]	10.0	50.0	100.0
Appl. lipid amount [mg]	1.00	5.00	10.00
Applied activity [cpm]	145599	727997	1E+06

5

To test the effect of changing administered dose per area over longer period of time, even greater suspension volumes were applied on upper back of test mice.

10

Resulting data are analysed and presented together with those from previous experimental series in figures 1 to 7.

Figure 1 shows the recovery of relative activity (penetrant amount) in different layers of the skin as a function of applied activity (dose).

15

Figure 2 shows the amount of carrier derived radioactivity (^3H -DPPC) in the blood as a function of time and epicutaneously administered penetrant quantity, expressed as percentage of applied dosage. As can be seen in this figure the relative amount of non-invasively administered lipid found in the blood reaches appreciable level after a clear lag-time of approximately 4 hours, but is nearly independent of the dose used.

20

Figure 3 indicates the relative accumulation of carrier derived radioactivity in various organs at two different time points after an increasing mass of ultradeformable carriers has been administered on the skin. It is apparent that whereas the relative amount of the carrier derived radioactivity decreases with the

25

applied dosage at both times of exploration, the phospholipid amount in the blood, viable skin and liver in parallel increases at $t = 8$ h, but remains nearly unchanged at $t = 24$ h.

5 Figure 4 shows the absolute penetrant distribution profile (in arbitrary units) in different layers of the skin as a function of applied activity (dose). Little dose dependence is seen in the horny layer for area doses between 0.5 mg cm^{-2} and up to 1.5 mg cm^{-2} , but greater penetrant amounts are deposited much more efficiently in the barrier. This is true 8 hours as well as 24 hours after the suspension
10 administration. Viable skin accumulates the penetrant derived material in a dose dependent fashion in entire investigated range.

Figure 5 shows the total amount of penetrant recovered in different tissues (skin, blood, liver) at different times after the administration of an increasing quantity of
15 ultradeformable penetrants on the skin grows with the applied dose per area. However, while at $t = 8$ h, an apparent saturation tendency is observed for doses greater than 1.5 mg cm^{-2} , at $t = 24$ h the dose dependence is linear.

Figure 6 shows the time dependence of penetrant derived radioactivity in the
20 blood as a function of epicutaneously administered suspension volume (lipid amount). As can be seen from this figure the temporal penetration characteristics are essentially independent of the applied dose: after a lag-time period of 4-6 hours, nearly steady state situation is observed.

25 Figure 7 shows the penetrant derived radioactivity in the blood as a function of epicutaneously administered dose measured 8 h or 24 h after the application. Linear extrapolation suggests that barrier starts to adapt itself to penetrant transport at approximately 0.75 mg cm^{-2} .

Non-occlusive one-compartment and multicompartment patches

Figure 8 shows the results obtained by measurement of the mean vapour transmission rate (MVTR) of five microporous polyethylene membranes, four
5 polyurethan membranes and one polycarbonate track etched membrane.

Abbreviations used:

First akronym:

DSM	DSM Solutech, Heerlen, The Netherlands
3M	3M Medica, Borken, Germany
10 ARCare	Adhesives Research, Limerick, Ireland
SM	Smith and Nephew
Infiltec	Infiltec, Speyer, Germany

Second akronym:

15 PE	microporous polyethylen
PU	polyurethan
PCTE	polycarbonate track etched

The third akronym refers to the article number.

Figure 9 is a diagram showing the principle of the "switching-effect", which e.g. is
20 observed in connection with the inventive hydrophobic mesh-membranes. A cross-section of two threads of a sieving material is given. In part 1 the threads are covered by a Transfersom®-formulation or lipid suspension without any contact to the skin, e.g. during storage. Contact with skin causes liquid bridges to the surface of the skin (part 2), which finally leads to complete skin wetting and
25 release of Transfersomes® through the "sieve" (part 3).

Figure 10 shows the penetrability of three different microporous polyethylen membranes for Transfersomes®, namely Type-C; Solupor - E011 D, Solupor - 8P07A and Solupor - 10P05A (DSM Solutech, Heerlen, The Netherlands). They

exhibit a high penetrability at small pressures thus allowing for Transfersomes to wet the skin upon contact. Moreover, it can be taken from the figure, that no penetration of the Transfersomes® through the membranes is observed, when the pressure is 0.

5

Figure 11 shows a schematic diagram of a multicompartment patch having external compartments according to the present invention in form of twin syringe serving as storage compartments with mixing tubing or T-piece connector attached to the patch.

10

Figure 12 shows a schematic diagram of a multicompartment patch according to the present invention having vertically stacked compartments.

15

Figure 13 shows a schematic diagram of a multicompartment patch according to the present invention with a side-by-side alignment of compartments with vertically introduced septum.

20

Figure 14 shows a schematic diagram of a multicompartment patch according to the present invention having a side-by-side alignment of compartments with separating lamination.

25

An example for a patch, which is suited for application of a Transfersome®-formulation ($V = 0.6\text{mL}$) according to the present invention is given below. Said transdermal patch can be used as an one-compartment patch according to the present invention and also can be fitted with external compartments thereby producing a multicompartment patch according to the present invention.

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Type	Material	Dimension
Backing liner	COTRAN 9701 / 3M 2 mil Polyurethan 70-0000-3993-6 SLP P261450106	Inner diameter 3.6 cm outer rectangle 4.5 cm *4.5 cm
Compartment	3M Foam tape 1779 polyolefin tape double layered # 70-0000-6467-8	
Inner liner	PCTE 100 nm Poretics, Cat 19410 LOT AE84AG11C024	
protective periphery	Leukoplast	
Injection tubing	Obturator Venflon 1.2 mm/18G L45 mm Art. No. 4253-1 LOT 931208	Preinstalled tubing; removed after TFS injection; port sealed with Leukoplast
Area of application	10 cm ²	
Application perimeter	3.6 cm	
Concentric seal width	> 0.8 cm	
Total area	20.25 cm ²	

Another example for a patch, which is suited for application of a Transfersome®-formulation according to the present invention is given below. Said patch has no inner liner membrane and is intended for direct application to the skin. Filling of

- 62 -

the mixing compartment (formed by the backing liner and the skin) can be done e.g. by external syringes connected to the mixing compartment.

Type	Material	Dimension
Backing liner	microporous Polyethylene 9711; 3M Medica #KG-90054	6 cm * 8.6 cm rectangle
Compartment	3M Foam tape 1779 polyolefin tape double layered # 70-0000-6467-8	outer rectangle 6 cm * 8.6 cm inner perimeter 4.4 cm * 7 cm
release cover I	from foam tape	
protective periphery	Leukoplast	
Injection tubing	Obturator Venflon 1.2 mm/18G L45 mm Art. No. 4253-1 LOT 931208	Preinstalled tubing; removed after TFS injection; port sealed with Leukoplast
Area of application	25 cm ²	
Application perimeter	4.4 cm * 7 cm	
Concentric seal width	> 0.8 cm	
Total area	51.6 cm ²	

CLAIMS

1. A method for controlling the flux of penetrants across an adaptable semi-permeable porous barrier comprising the steps of:
- 5 – preparing a formulation by suspending or dispersing said penetrants in a polar liquid in the form of fluid droplets surrounded by a membrane-like coating of one or several layers, said coating comprising at least two kinds or forms of amphiphilic substances with a tendency to aggregate, provided that
 - 10 – said at least two substances differ by at least a factor of 10 in solubility in said polar liquid,
 - and / or said substances when in the form of homo-aggregates (for the more soluble substance) or of hetero-aggregates (for any combination of both said substances) have a preferred average diameter smaller than the diameter of
 - 15 homo-aggregates containing merely the less soluble substance,
 - and / or the more soluble substance tends to solubilise the droplet and the content of such substance is to up to 99 mol-% of solubilising concentration or else corresponds to up to 99 mol-% of the saturating concentration in the unsolubilised droplet, whichever is higher;
 - 20 – and / or the presence of the more soluble substance lowers the average elastic energy of the membrane-like coating to a value at least 5 times lower, more preferably at least 10 times lower and most preferably more than 10 times lower, than the average elastic energy of red blood cells or of phospholipid bilayers with fluid aliphatic chains,
 - 25 – said penetrants being able to transport agents through the pores of said barrier or to enable agent permeation through the pores of said barrier after penetrants have entered the pores,
 - selecting a dose amount of said penetrants to be applied on a predetermined area of said barrier to control the flux of said penetrants across said barrier, and

- applying the selected dose amount of said formulation containing said penetrants onto said area of said porous barrier.

2. The method according to claim 1,
5 **characterised in that** the flux across said barrier is increased by enlarging the applied dose per area of said penetrants.

3. The method according to claims 1 or 2,
characterised in that the pH of the formulation is between 3 and 10, more
10 preferably between 4 and 9, and most preferably between 5 and 8.

4. The method according to any one of the preceding claims,
characterised in that the formulation comprises:
- at least one thickening agent in an amount that increases the formulation
15 viscosity to maximally 5 Nm/s, more preferably up to 1 Nm/s, and most preferably up to 0.2 Nm/s, so that formulation spreading-over, and drug retention at the application area is enabled,
- and / or at least one antioxidant in an amount that reduces the increase of
oxidation index to less than 100 % per 6 months, more preferably to less than
20 100 % per 12 months and most preferably to less than 50 % per 12 months
- and / or at least one microbicide in an amount that reduces the bacterial count of 1 million germs added per g of total mass of the formulation to less than 100 in the case of aerobic bacteria, to less than 10 in the case of entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa* or *Staphylococcus aureus*,
25 after a period of 4 days.

5. The method according to claim 4,
characterised in that said at least one microbicide is added in an amount that reduces the bacterial count of 1 million germs added per g of total mass of the

formulation to less than 100 in the case of aerobic bacteria, to less than 10 in the case of entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa* or *Staphylococcus aureus*, after a period of 3 days, and more preferably after a period of 1 day.

5

6. The method according to claim 4,
characterised in that said thickening agent is selected from the class of pharmaceutically acceptable hydrophilic polymers, such as partially etherified cellulose derivatives, like carboxymethyl-, hydroxyethyl-, hydroxypropyl-,
10 hydroxypropylmethyl- or methyl-cellulose; completely synthetic hydrophilic polymers such as polyacrylates, polymethacrylates, poly(hydroxyethyl)-, poly(hydroxypropyl)-, poly(hydroxypropylmethyl)methacrylates, polyacrylonitriles, methallyl-sulphonates, polyethylenes, polyoxiethylenes, polyethylene glycols, polyethylene glycol-lactides, polyethylene glycol-diacrylates,
15 polyvinylpyrrolidones, polyvinyl alcohols, poly(propylmethacrylamides), poly(propylene fumarate-co-ethylene glycols), poloxamers, polyaspartamides, (hydrazine cross-linked) hyaluronic acids, silicones; natural gums comprising alginates, carrageenans, guar-gums, gelatines, tragacanth, (amidated) pectins, xanthans, chitosan collagens, agaroses; mixtures and further derivatives or
20 co-polymers thereof and / or other pharmaceutically, or at least biologically, acceptable polymers.

7. The method according to claim 6,
characterised in that the concentration of said polymer is in the range between
25 0.01 w- % and 10 w- %, more preferably in the range between 0.1 w- % and 5 w- %, even more preferably in the range between 0.25 w- % and 3.5 w- % and most preferably in the range between 0.5 w- % and 2 w- %.

8. The method according to claim 4,

characterised in that said anti-oxidant is selected from synthetic phenolic antioxidants, such as butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT) and di-tert-butylphenol (LY178002, LY256548, HWA-131, BF-389, CI-986, PD-127443, E-5119, BI-L-239XX, etc.), tertiary butylhydroquinone (TBHQ),
5 propyl gallate (PG), 1-O-hexyl-2,3,5-trimethylhydroquinone (HTHQ); aromatic amines (such as diphenylamine, p-alkylthio-o-anisidine, ethylenediamine derivatives, carbazol, tetrahydroindenoindol); phenols and phenolic acids (such as guaiacol, hydroquinone, vanillin, gallic acids and their esters, protocatechuic acid, quinic acid, syringic acid, ellagic acid, salicylic acid, nordihydroguaiaretic acid
10 (NDGA), eugenol); tocopherols (including tocopherols (alpha, beta, gamma, delta) and their derivatives, such as tocopheryl-acylate (e.g. -acetate, -laurate, myristate, -palmitate, -oleate, -linoleate, etc., or any other suitable tocopheryl-lipoate), tocopheryl-POE-succinate; trolox and corresponding amide- and thiocarboxamide analogues; ascorbic acid and its salts, isoascorbate, (2 or 3 or 6)-o-alkylascorbic
15 acids, ascorbyl esters (e.g. 6-o-lauroyl, myristoyl, palmitoyl-, oleoyl, or linoleoyl-L-ascorbic acid, etc.); non-steroidal anti-inflammatory agents (NSAIDs), such as indomethacin, diclofenac, mefenamic acid, flufenamic acid, phenylbutazone, oxyphenbutazone acetylsalicylic acid, naproxen, diflunisal, ibuprofen, ketoprofen, piroxicam, penicillamine, penicillamine disulphide,
20 primaquine, quinacrine, chloroquine, hydroxychloroquine, azathioprine, phenobarbital, acetaminophen); aminosalicylic acids and derivatives; methotrexate, probucol, antiarrhythmics (e.g. amiodarone, aprindine, asocainol), ambroxol, tamoxifen, b-hydroxytamoxifen; calcium antagonists (such as nifedipine, nisoldipine, nimodipine, nicardipine, nilvadipine), beta-receptor
25 blockers (e.g. atenolol, propranolol, nebivolol); sodium bisulphite, sodium metabisulphite, thiourea; chelating agents, such as EDTA, GDTA, desferral; endogenous defence systems, such as transferrin, lactoferrin, ferritin, ceruloplasmin, haptoglobin, haemopexin, albumin, glucose, ubiquinol-10; enzymatic antioxidants, such as superoxide dismutase and metal complexes with a

similar activity, including catalase, glutathione peroxidase, and less complex molecules, such as beta-carotene, bilirubin, uric acid; flavonoids (e.g. flavones, flavonols, flavonones, flavanonals, chalcones, anthocyanins), N-acetylcystein, mesna, glutathione, thiohistidine derivatives, triazoles; tannines, cinnamic acid, hydroxycinnamic acids and their esters (e.g. coumaric acids and esters, caffeic acid and their esters, ferulic acid, (iso-) chlorogenic acid, sinapic acid); spice extracts (e.g. from clove, cinnamon, sage, rosemary, mace, oregano, allspice, nutmeg); carnosic acid, carnosol, carsolic acid; rosmarinic acid, rosmarindiphenol, gentisic acid, ferulic acid; oat flour extracts, such as avenanthramide 1 and 2; thioethers, dithioethers, sulfoxides, tetraalkylthiuram disulphides; phytic acid, steroid derivatives (e.g. U74006F); tryptophan metabolites (e.g. 3-hydroxykynurenine, 3-hydroxyanthranilic acid), and organochalcogenides, or else is an oxidation suppressing enzyme.

15 9. The method according to claim 8,
characterised in that the concentration of BHA or BHT is between 0.001 and 2 w-%, more preferably is between 0.0025 and 0.2 w-%, and most preferably is between 0.005 and 0.02 w-%, of TBHQ and PG is between 0.001 and 2 w-%, more preferably is between 0.005 and 0.2 w-%, and most preferably is between 0.01 and 0.02 w-%, of tocopherols is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.075 w-%, of ascorbic acid esters is between 0.001 and 5, more preferably is between 0.005 and 0.5, and most preferably is between 0.01 and 0.15 w-%, of ascorbic acid is between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01 and 0.1 w-%, of sodium bisulphite or sodium metabisulphite is between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01-0.15 w-%, of thiourea is between 0.0001 and 2 w-%, more preferably is between 0.0005 and 0.2, and most preferably is between 0.001-0.01 w-%, most typically 0.005 w-%, of cystein is

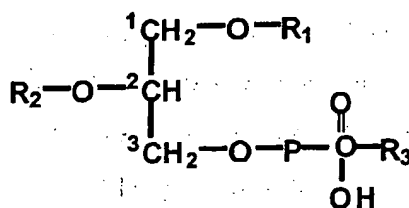
between 0.01 and 5, more preferably is between 0.05 and 2 w-%, and most preferably is between 0.1 and 1.0 w-%, most typically 0.5 w-%, of monothioglycerol is between 0.01 and 5 w-%, more preferably is between 0.05 and 2 w-%, and most preferably is between 0.1-1.0 w-%, most typically 0.5 w-%, of NDGA is between 0.0005-2 w-%, more preferably is between 0.001-0.2 w-%, and most preferably is between 0.005-0.02 w-%, most typically 0.01 w-%, of glutathione is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.2 w-%, most typically 0.1 w-%, of EDTA is between 0.001 and 5 w-%, even more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01 and 0.2 w-%, most typically between 0.05 and 0.975 w-%, of citric acid is between 0.001 and 5 w-%, even more preferably is between 0.005 and 3 w-%, and most preferably is between 0.01-0.2, most typically between 0.3 and 2 w-%.

10. The method according claim 4,
characterised in that said microbicide is selected from short chain alcohols, such as ethyl and isopropyl alcohol, chlorbutanol, benzyl alcohol, chlorbenzyl alcohol, dichlorbenzylalcohol; hexachlorophene; phenolic compounds, such as cresol, 4-chloro-m-cresol, p-chloro-m-xenol, dichlorophene, hexachlorophene, povidon-iodine; parabens, especially alkyl-paraben, such as methyl-, ethyl-, propyl-, or butyl-paraben, benzyl-paraben; acids, such as sorbic acid, benzoic acid and its salts; quaternary ammonium compounds, such as alkonium salts, e.g. benzalkonium salts, especially the chlorides or bromides, cetrimonium salts, e.g. the bromide; phenoalkecinium salt, such as phenododecinium bromide, cetylpyridinium chloride or other such salts; mercurium compounds, such as phenylmercuric acetate, borate, or nitrate, thiomersal; chlorhexidine or its gluconate; antibiotically active compounds of biological origin, or a mixture thereof.

11. The method according to claim 10,
characterised in that the bulk concentration of short chain alcohols in the case of ethyl, propyl, butyl or benzyl alcohol is up to 10 w-%, more preferably is up to 5 w-%, and most preferably is in the range between 0.5-3 w-%, and in the case of
5 chlorobutanol is in the range between 0.3-0.6 w-%; bulk concentration of parabens, especially in the case of methyl paraben is in the range between 0.05-0.2 w-%, and in the case of propyl paraben is in the range between 0.002-0.02 w-%; bulk concentration of sorbic acid is in the range between 0.05-0.2 w-%, and in the case of benzoic acid is in the range between 0.1-0.5 w-%;
10 bulk concentration of phenols, triclosan, is in the range between 0.1-0.3 w-%, and bulk concentration of chlorhexidine is in the range between 0.01-0.05 w-%.

12. The method according to any one of the preceding claims,
15 **characterised in that** the less soluble amongst the aggregating substances is a lipid or lipid-like material, especially a polar lipid, whereas the substance which is more soluble in the suspending liquid and which lowers the average elastic energy of the droplet is a surfactant or else has surfactant-like properties and / or is a form of said lipid or lipid-like material which is comparably as soluble as said
20 surfactant or the surfactant-like material.

13. The method according to claim 12,
characterised in that the lipid or lipid-like material is a lipid or a lipoid from a biological source or a corresponding synthetic lipid or any of its modifications,
25 said lipid preferably belonging to the class of pure phospholipids corresponding to the general formula



where R_1 and R_2 is an aliphatic chain, typically a C_{10-20} -acyl, or -alkyl or partly unsaturated fatty acid residue, in particular, an oleoyl-, palmitoeloyl-, elaidoyl-,
 5 linoleyl-, linolenyl-, linolenoyl-, arachidoyl-, vaccinyl-, lauroyl-, myristoyl-, palmitoyl-, or stearoyl chain; and where R_3 is hydrogen, 2-trimethylamino-1-ethyl, 2-amino-1-ethyl, C_{1-4} -alkyl, C_{1-5} -alkyl substituted with carboxy, C_{2-5} -alkyl substituted with hydroxy, C_{2-5} -alkyl substituted with carboxy and hydroxy, or C_{2-5} -alkyl substituted with carboxy and amino, inositol, sphingosine, or salts of said
 10 substances, said lipid comprising also glycerides, isoprenoid lipids, steroids, sterines or sterols, of sulphur- or carbohydrate-containing lipids, or any other bilayer-forming lipids, in particular half-protonated fluid fatty acids, said lipid is selected from the group comprising phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositols,
 15 phosphatidic acids, phosphatidylserines, sphingomyelins or other sphingophospholipids, glycosphingolipids (including cerebrosides, ceramidepolyhexosides, sulphatides, sphingoplasmalogens), gangliosides and other glycolipids or synthetic lipids, in particular with corresponding sphingosine derivatives, or any other glycolipids, whereby two similar or different chains can
 20 be ester-groups-linked to the backbone (as in diacyl and dialkenoyl compound) or be attached to the backbone with ether bonds, as in dialkyl-lipids.

14. The method according to claim 12,

characterised in that the surfactant or surfactant-like material is a nonionic, a zwitterionic, an anionic or a cationic surfactant, especially a fatty-acid or -alcohol, an alkyl-tri/di/methyl-ammonium salt, an alkylsulphate salt, a monovalent salt of cholate, deoxycholate, glycocholate, glycodeoxycholate, taurodeoxycholate, taurocholate, etc., an acyl- or alkanoyl-dimethyl- aminoxide, esp. a dodecyl-
5 dimethyl-aminoxide, an alkyl- or alkanoyl-N-methylglucamide, N- alkyl-N,N-dimethylglycine, 3-(acyldimethylammonio)-alkanesulphonate, N-acyl-sulphobetaine, a polyethylene-glycol-octylphenyl ether, esp. a nonaethylene-glycol-octylphenyl ether, a polyethylene-acyl ether, esp. a nonaethylen-dodecyl
10 ether, a polyethylene-glycol-isoacyl ether, esp. a octaethylene-glycol-isotridecyl ether, polyethylene-acyl ether, esp. octaethylenedodecyl ether, polyethylene-glycol-sorbitane-acyl ester, such as polyethylenglykol-20-monolaurate (Tween 20) or polyethylenglykol-20-sorbitan-monooleate (Tween 80), a polyhydroxyethylene-acyl ether, esp. polyhydroxyethylene- lauryl, -myristoyl, -cetylstearyl, or -oleoyl
15 ether, as in polyhydroxyethylene-4 or 6 or 8 or 10 or 12, etc., -lauryl ether (as in Brij series), or in the corresponding ester, e.g. of polyhydroxyethylen-8-stearate (Myrj 45), -laurate or -oleate type, or in polyethoxylated castor oil 40, a sorbitane-monoalkylate (e.g. in Arlacel or Span), esp. sorbitane-monolaurate, an acyl- or alkanoyl-N-methylglucamide, esp. in or decanoyl- or dodecanoyl-N-
20 methylglucamide, an alkyl-sulphate (salt), e.g. in lauryl- or oleoyl-sulphate, sodium deoxycholate, sodium glycodeoxycholate, sodium oleate, sodium taurate, a fatty acid salt, such as sodium elaidate, sodium linoleate, sodium laurate, a lysophospholipid, such as n-octadecylene(=oleoyl)-glycerophosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-acyl-, e.g. lauryl or oleoyl-glycero-
25 phosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-tetradecyl-glycero-phosphatidic acid, -phosphorylglycerol, or - phosphorylserine, a corresponding palmitoeloyl-, elaidoyl-, vaccenyl-lysophospholipid or a corresponding short-chain phospholipid, or else a surface-active polypeptide.

15. The method according to any of the preceding claims,
characterised in that the average diameter of the penetrant is between 30 nm and
500 nm, more preferably between 40 nm and 250 nm, even more preferably
5 between 50 nm and 200 nm and particularly preferably between 60 nm and
150 nm.

16. The method according to any one of the preceding claims,
characterised in that the total dry weight of droplets in a formulation is
10 0.01 weight-% (w-%) to 40 w-% of total formulation mass, more preferably
between 0.1 w-% and 30 w-%, and most preferably between 0,5 w-% and 20 w-%.

17. The method according to any one of the preceding claims,
characterised in that the total dry weight of droplets in a formulation is selected
15 to increase the formulation viscosity to maximally 200 mPas, more preferably up
to 40 mPas, and most preferably up to 8 mPas.

18. The method according to any one of the preceding claims,
characterised in that at least one amphiphilic substance and/or at least one edge-
20 active substance or surfactant, and / or at least one hydrophilic fluid and the agent
are mixed, if required separately, to form a solution, the resulting (partial)
mixtures or solutions are then combined subsequently to induce, preferably by
action of mechanical energy such as shaking, stirring, vibrations, homogenisation,
ultrasonication, shearing, freezing and thawing, or filtration using convenient
25 driving pressure, the formation of penetrants that associate with and / or
incorporate the agent

19. The method of claim 18;

characterised in that said amphiphilic substances are dissolved in volatile solvents, such as alcohols, especially ethanol, or in other pharmaceutically acceptable organic solvents, such as ethanol, 1- and 2-propanol, benzyl alcohol, propylene glycol, polyethylene glycol (molecular weight: 200-400 D) or glycerol, other pharmaceutically acceptable organic solvents, such as undercooled gas, especially supercritical CO₂, which are then removed, especially by evaporation or dilution, prior to making the final preparation.

20. The method according to any one of claims 18 or 19, characterised in that the formation of said penetrants is induced by the addition of required substances into a fluid phase, evaporation from a reverse phase, by injection or dialysis, if necessary under the influence of mechanical stress, such as shaking, stirring, in especially high velocity stirring, vibrating, homogenising, ultrasonication, shearing, freezing and thawing, or filtration using convenient, in especially low (1 MPa) or intermediate (up to 10 MPa), driving pressure.

21. The method of claim 20, characterised in that the formation of said penetrants is induced by filtration, the filtering material having pores sizes between 0.01 µm and 0.8 µm, more preferably between 0.02 µm and 0.3 µm, and most preferably between 0.05 µm and 0.15 µm, whereby several filters may be used sequentially or in parallel.

22. The method according to any one of claims 18 to 21, characterised in that said agents and penetrants are made to associate, at least partly,

– after the formation of said penetrants, e.g. after injecting a solution of the drug in a pharmaceutically acceptable fluid, such as ethanol, 1- and 2-propanol, benzyl alcohol, propylene glycol, polyethylene glycol (molecular weight: 200-400 D) or glycerol into the suspending medium,

- simultaneously with penetrant formation, if required using the drug co-solution and, at least some, penetrant ingredients.

23. The method according to any one of the claims 18 to 22,
5 **characterised in that** said penetrants, with which the agent is associated, are prepared immediately before the application of the formulation, if convenient, from a suitable concentrate or a lyophilisate.

24. The method according to any one of the preceding claims,
10 **characterised in that** the formulation is applied by spraying, smearing, rolling or sponging on the application area, in particular by using a metering sprayer, spender, roller, sponge or a non-occlusive patch, as appropriate.

25. The method according to any one of the preceding claims,
15 **characterised in that** the barrier is a part of a mammalian body and / or a plant and preferably is skin and / or at least partly keratinised endothelium and / or nasal or any other mucosa.

26. The method according to claim 25,
20 **characterised in that**, the area dose of said penetrant is between 0.1 mg per square centimetre (mg cm^{-2}) and 40 mg cm^{-2} , more preferably is between 0.25 mg cm^{-2} and 30 mg cm^{-2} and even more preferably is between 0.5 mg cm^{-2} and 15 mg cm^{-2} , in case the penetrant is applied on said skin and / or said at least partly keratinised endothelium.

25

27. The method according to claim 25,
characterised in that the area dose of said penetrant is between 0.05 mg per square centimetre (mg cm^{-2}) and 20 mg cm^{-2} , more preferably is between 0.1 mg cm^{-2} and 15 mg cm^{-2} and even more preferably is between 0.5 mg cm^{-2}

and 10 mg cm^{-2} , in the case the penetrant is applied on said nasal or other mucosa.

28. The method according to claim 25,
5 **characterised in that** the area dose of said penetrant is between 0.0001 mg per square centimetre (mg cm^{-2}) and 0.1 mg cm^{-2} , more preferably is between $0.0005 \text{ mg cm}^{-2}$ and 0.05 mg cm^{-2} and even more preferably is between 0.001 mg cm^{-2} and 0.01 mg cm^{-2} , in the case that the penetrant is applied on plant body, plant leaves or plant needles.

10

29. A kit containing said formulation in an amount which enables the formulation to be applied at the selected dose per area, according to any one of the preceding claims.

15

30. The kit according to claim 29,
characterised in that the formulation is contained in a bottle or any other packaging vessel.

20

31. The kit according to claims 29 or 30,
characterised in that it contains a device for administering the formulation.

25

32. A patch, containing the formulation as in any one of claims 1 to 28 in an amount that yields the dose per area according to any one of the preceding claims.

33. The patch according to claim 32,
comprising:
— a non-occlusive backing liner;

- an inner liner, wherein the backing liner and the inner liner define a reservoir; and /or a matrix layer.

34. The patch according to claims 32 or 33,
5 **characterised in that** the non-occlusive backing liner exhibits a mean vapor transmission rate (MVTR) of more than 1000 g/m²day, preferably of more than 5.000 g/m²day and most preferably of more than 10.000 g/m²day.

35. The patch according to claims 32 or 34,
10 **characterised in that** the non-occlusive backing liner has pores of smaller than 100 nm, preferably smaller than 70 nm and most preferably of smaller than 30 nm.

36. The patch according to any one of claims 32 to 35,
15 **characterised in that** the non-occlusive backing liner comprises a polyurethane membrane, preferably a polyester track-etched porous membrane, more preferably a polycarbonate track-etched porous membrane and most preferably a polyethylene microporous membrane.

37. The patch according to any one of claims 32 to 36,
20 **characterised in that** the inner liner prevents unwanted release of the formulation from the patch during storage and enables rapid skin wetting when contacted with the skin.

38. The patch according to any one of claims 32 to 37,
25 **characterised in that** the inner liner comprises a homogeneous membrane, preferably a polyester track-etched porous membrane or a polycarbonate track-etched porous membrane.

39. The patch according to claim 38,

characterised in that the membranes have a pore density of up to 5%, preferably of up to 15%, more preferably of up to 25% and most preferably of more than 25% and/or a pore size in the range between 20 nm and 200 nm, preferably between 50 nm and 140 nm and most preferably between 80 nm and 120 nm.

5

40. The patch according to any one of claims 32 to 39,
characterised in that the inner liner comprises a hydrophobic mesh-membrane and/or a nonwoven fleece with mesh openings formed by hydrophobic threads.

10

41. The patch according to any one of claims 32 to 40,
characterised in that the inner liner comprises a microporous polyethylene membrane having average pore sizes in the range of between 50 nm to 3000 nm, preferably between 500 nm to 2000 nm and most preferably of about 1500 nm.

15

42. The patch according to any one of claims 32 to 41,
characterised in that the patch comprises a pressure sensitive adhesive layer, preferably an adhesive layer comprising polyacrylate, polyisobutylene, silicone, ethylene vinyl acetate copolymer, polyvinylpyrrolidone or polyethylene oxide hydrogel.

20

43. The patch according to any one of claims 32 to 42,
characterised in that the formulation viscosity is up to maximally 200 mPas, more preferably up to 40 mPas, and most preferably up to 8 mPas.

25

44. The patch according to any one of claims 32 to 43,
characterised in that the patch comprises one or more additional layers comprising desiccant containing layers, matrix layers, foam tape layers and/or protective layers.

45. The patch according to claim 32 to 44,
characterised in that the patch comprises at least two compartments, which are separated from each other during storage.

5 46. The patch according to claim 32 to 45,
characterised in that at least one of the compartments is inside and / or outside the patch.

10 47. The patch according to claim 32 to 46,
characterised in that the formulation and / or the individual formulation components and/or the agent and / or the suspension / dispersion of penetrants without the agent are kept during the storage in several, preferably less than 5, more preferably in 3, and most preferred in 2 separate compartments of the patch which, in case, are combined prior to or during or after the application of the
15 patch.

 48. The patch according to claim 32 to 47,
characterised in that the outer compartment(s) comprise(s) injection systems, which are connected to the reservoir.

20 49. The patch according to claim 32 to 47,
characterised in that the compartments are inside the reservoir, which is defined by the backing liner and the inner liner.

25 50. The patch according to claim 32 to 47,
characterised in that the compartments are vertically stacked and /or are arranged side-by-side and / or one compartment is included in a second compartment, preferably without being fixed to the second compartment.

51. The patch according to claim 49 or 50,
characterised in that the compartments are separated from each other by a
controllably openable barrier, preferably a membrane and /or by a plug and / or by
a compartment-forming lamination.

5

52. The patch according to claim 45 to 51,
characterised in that combining and mixing of the ingredients of the
compartments is achieved by direct mechanical action, such as pressing, rubbing,
kneading, twisting, tearing and /or indirectly by changing the temperature, osmotic
pressure or electrical potential.

10

53. The patch according to claim 32,
comprising:

- a non-occlusive backing liner as in any of claims 34 to 37
- a membrane defining a reservoir, which is divided in at least two
compartments,

15

characterised in that the formulation directly contacts the skin when the
formulation releases from the reservoir.

20

54. A method of administering an agent to a mammalian body or a
plant, by transporting said agent through a barrier, wherein the barrier is the intact
skin, mucosa and/or cuticle of said mammalian body or a plant, said agent being
associated to a penetrant capable of transporting said agent through the skin pores
or through the passages in mucosa or cuticle, or capable of enabling agent
permeation through skin pores after said penetrant has opened and/or entered said
pores, comprising the steps of:

25

- preparing a formulation by suspending or dispersing said penetrants in a polar
liquid in the form of fluid droplets surrounded by a membrane-like coating of

one or several layers, said coating comprising at least two kinds or forms of amphiphilic substances with a tendency to aggregate, provided that

– said at least two substances differ by at least a factor of 10 in solubility in said polar liquid,

5 – and / or said substances when in the form of homo-aggregates (for the more soluble substance) or of hetero-aggregates (for any combination of both said substances) have a preferred average diameter smaller than the diameter of homo-aggregates containing merely the less soluble substance,

– and / or the more soluble substance tends to solubilise the droplet and the
10 content of such substance is to up to 99 mol-% of solubilising concentration or else corresponds to up to 99 mol-% of the saturating concentration in the unsolubilised droplet, whichever is higher,

– and / or the presence of the more soluble substance lowers the average elastic energy of the membrane-like coating to a value at least 5 times lower, more
15 preferably at least 10 times lower and most preferably more than 10 times lower, than the average elastic energy of red blood cells or of phospholipid bilayers with fluid aliphatic chains,

– said penetrants being able to transport agents through the pores of said barrier or being able to promote agent permeation through the pores of said skin after
20 penetrants have entered the pores,

– selecting a dose amount of said penetrants to be applied on a predetermined area of said barrier to control the flux of said penetrants across said barrier, and

– applying the selected dose amount of said formulation containing said penetrants onto said area of said porous barrier.

25

55. The method according to claim 54,
characterised in that the flux of penetrants across said barrier is increased by enlarging the applied dose per area of said penetrants.

56. The method according to claims 54 or 45,
characterised in that the pH of the formulation is between 3 and 10, more preferably between 4 and 9, and most preferably between 5 and 8.

5 57. The method according to claims 54 to 56,
characterised in that the formulation comprises:

- at least one thickening agent in an amount that increases the formulation viscosity to maximally 5 Nm/s, more preferably up to 1 Nm/s, and most preferably up to 0.2 Nm/s, so that formulation spreading-over, and drug
10 retention at the application area is enabled,
- and / or at least one antioxidant in an amount that reduces the increase of oxidation index to less than 100 % per 6 months, more preferably to less than 100 % per 12 months and most preferably to less than 50 % per 12 months
- and / or at least one microbicide in an amount that reduces the bacterial count
15 of 1 million germs added per g of total mass of the formulation to less than 100 in the case of aerobic bacteria, to less than 10 in the case of entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa* or *Staphylococcus aureus*, after a period of 4 days.

20 58. Method according to claim 54,
characterised in that said at least one microbicide is added in an amount that reduces the bacterial count of 1 million germs added per g of total mass of the formulation to less than 100 in the case of aerobic bacteria, to less than 10 in the case of entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa*
25 or *Staphylococcus aureus*, after a period of 3 days, and more preferably after a period of 1 day.

59. The method according to claim 54,

characterised in that said thickening agent is selected from the class of pharmaceutically acceptable hydrophilic polymers, such as partially etherified cellulose derivatives, like carboxymethyl-, hydroxyethyl-, hydroxypropyl-, hydroxypropylmethyl- or methyl-cellulose; completely synthetic hydrophilic polymers such as polyacrylates, polymethacrylates, poly(hydroxyethyl)-, poly(hydroxypropyl)-, poly(hydroxypropylmethyl)methacrylates, polyacrylonitriles, methallyl-sulphonates, polyethylenes, polyoxiethylenes, polyethylene glycols, polyethylene glycol-lactides, polyethylene glycol-diacrylates, polyvinylpyrrolidones, polyvinyl alcohols, poly(propylmethacrylamides), poly(propylene fumarate-co-ethylene glycols), poloxamers, polyaspartamides, (hydrazine cross-linked) hyaluronic acids, silicones; natural gums comprising alginates, carrageenans, guar-gums, gelatines, tragacanth, (amidated) pectins, xanthans, chitosan collagens, agaroses; mixtures and further derivatives or co-polymers thereof and / or other pharmaceutically, or at least biologically, acceptable polymers.

60. The method according to claim 59, characterised in that the concentration of said polymer is in the range between 0.01 w- % and 10 w- %, more preferably in the range between 0.1 w- % and 5 w- %, even more preferably in the range between 0.25 w- % and 3.5 w- % and most preferably in the range between 0.5 w- % and 2 w- %.

61. The method according to claim 54, characterised in that said anti-oxidant is selected from synthetic phenolic antioxidants, such as butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT) and di-tert-butylphenol (LY178002, LY256548, HWA-131, BF-389, CI-986, PD-127443, E-5119, BI-L-239XX, etc.), tertiary butylhydroquinone (TBHQ), propyl gallate (PG), 1-O-hexyl-2,3,5-trimethylhydroquinone (HTHQ); aromatic amines (such as diphenylamine, p-alkylthio-o-anisidine, ethylenediamine

derivatives, carbazol, tetrahydroindenoindol); phenols and phenolic acids (such as guaiacol, hydroquinone, vanillin, gallic acids and their esters, protocatechuic acid, quinic acid, syringic acid, ellagic acid, salicylic acid, nordihydroguaiaretic acid (NDGA), eugenol); tocopherols (including tocopherols (alpha, beta, gamma, delta) and their derivatives, such as tocopheryl-acylate (e.g. -acetate, -laurate, myristate, 5 -palmitate, -oleate, -linoleate, etc., or any other suitable tocopheryl-lipoate), tocopheryl-POE-succinate; trolox and corresponding amide- and thiocarboxamide analogues; ascorbic acid and its salts, isoascorbate, (2 or 3 or 6)-o-alkylascorbic acids, ascorbyl esters (e.g. 6-o-lauroyl, myristoyl, palmitoyl-, oleoyl, or 10 -linoleoyl-L-ascorbic acid, etc.); non-steroidal anti-inflammatory agents (NSAIDs), such as indomethacin, diclofenac, mefenamic acid, flufenamic acid, phenylbutazone, oxyphenbutazone acetylsalicylic acid, naproxen, diflunisal, ibuprofen, ketoprofen, piroxicam, penicillamine, penicillamine disulphide, primaquine, quinacrine, chloroquine, hydroxychloroquine, azathioprine, 15 phenobarbital, acetaminophen); aminosalicyclic acids and derivatives; methotrexate, probucol, antiarrhythmics (e.g. amiodarone, aprindine, asocainol), ambroxol, tamoxifen, b-hydroxytamoxifen; calcium antagonists (such as nifedipine, nisoldipine, nimodipine, nicardipine, nilvadipine), beta-receptor blockers (e.g. atenolol, propranolol, nebivolol); sodium bisulphite, sodium 20 metabisulphite, thiourea; chelating agents, such as EDTA, GDTA, desferral; endogenous defence systems, such as transferrin, lactoferrin, ferritin, ceruloplasmin, haptoglobin, haemopexin, albumin, glucose, ubiquinol-10; enzymatic antioxidants, such as superoxide dismutase and metal complexes with a similar activity, including catalase, glutathione peroxidase, and less complex 25 molecules, such as beta-carotene, bilirubin, uric acid; flavonoids (e.g. flavones, flavonols, flavonones, flavanonals, chalcones, anthocyanins), N-acetylcystein, mesna, glutathione, thiohistidine derivatives, triazoles; tannines, cinnamic acid, hydroxycinnamic acids and their esters (e.g. coumaric acids and esters; caffeic acid and their esters, ferulic acid, (iso-) chlorogenic acid, sinapic acid); spice

extracts (e.g. from clove, cinnamon, sage, rosemary, mace, oregano, allspice, nutmeg); carnosic acid, carnosol, carsolic acid; rosmarinic acid, rosmarindiphenol, gentisic acid, ferulic acid; oat flour extracts, such as avenanthramide 1 and 2; thioethers, dithioethers, sulphoxides, tetralkylthiuram disulphides; phytic acid, steroid derivatives (e.g. U74006F); tryptophan metabolites (e.g. 3-hydroxykynurenine, 3-hydroxyanthranilic acid), and organochalcogenides, or else is an oxidation suppressing enzyme.

62. The method according to claim 54, characterised in that the concentration of BHA or BHT is between 0.001 and 2 w-%, more preferably is between 0.0025 and 0.2 w-%, and most preferably is between 0.005 and 0.02 w-%, of TBHQ and PG is between 0.001 and 2 w-%, more preferably is between 0.005 and 0.2 w-%, and most preferably is between 0.01 and 0.02 w-%, of tocopherols is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.075 w-%, of ascorbic acid esters is between 0.001 and 5, more preferably is between 0.005 and 0.5, and most preferably is between 0.01 and 0.15 w-%, of ascorbic acid is between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01 and 0.1 w-%, of sodium bisulphite or sodium metabisulphite is between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01-0.15 w-%, of thiourea is between 0.0001 and 2 w-%, more preferably is between 0.0005 and 0.2, and most preferably is between 0.001-0.01 w-%, most typically 0.005 w-%, of cystein is between 0.01 and 5, more preferably is between 0.05 and 2 w-%, and most preferably is between 0.1 and 1.0 w-%, most typically 0.5 w-%, of monothioglycerol is between 0.01 and 5 w-%, more preferably is between 0.05 and 2 w-%, and most preferably is between 0.1-1.0 w-%, most typically 0.5 w-%, of NDGA is between 0.0005-2 w-%, more preferably is between 0.001-0.2 w-%, and most preferably is between 0.005-0.02 w-%, most typically 0.01 w-%, of

glutathione is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.2 w-%, most typically 0.1 w-%, of EDTA is between 0.001 and 5 w-%, even more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01 and 0.2 w-%, most typically between 0.05 and 0.975 w-%, of citric acid is between 0.001 and 5 w-%, even more preferably is between 0.005 and 3 w-%, and most preferably is between 0.01-0.2, most typically between 0.3 and 2 w-%.

63. The method according claim 54,
characterised in that said microbicide is selected amongst short chain alcohols, such as ethyl and isopropyl alcohol, chlorbutanol, benzyl alcohol, chlorbenzyl alcohol, dichlorbenzylalcohol; hexachlorophene; phenolic compounds, such as cresol, 4-chloro-m-cresol, p-chloro-m-xylene, dichlorophene, hexachlorophene, povidon-iodine; parabens, especially alkyl-paraben, such as methyl-, ethyl-, propyl-, or butyl-paraben, benzyl-paraben; acids, such as sorbic acid, benzoic acid and its salts; quaternary ammonium compounds, such as alkonium salts, e.g. benzalkonium salts, especially the chlorides or bromides, cetrimonium salts, e.g. the bromide; phenoalkecinium salt, such as phenododecinium bromide, cetylpyridinium chloride or other such salts; mercurium compounds, such as phenylmercuric acetate, borate, or nitrate, thiomersal; chlorhexidine or its gluconate; antibiotically active compounds of biological origin, or a mixture thereof.

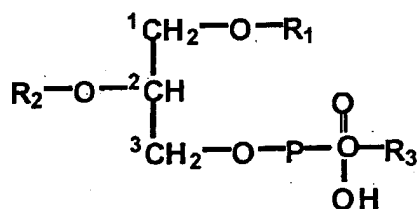
64. The method according claim 63,
characterised in that the bulk concentration of short chain alcohols in the case of ethyl, propyl, butyl or benzyl alcohol is up to 10 w-%, more preferably is up to 5 w-%, and most preferably is in the range between 0.5-3 w-%, and in the case of chlorobutanol is in the range between 0.3-0.6 w-%; bulk concentration of parabens, especially in the case of methyl paraben is in the range between

0.05-0.2 w-%, and in the case of propyl paraben is in the range between
 0.002-0.02 w-%; bulk concentration of sorbic acid is in the range between 0
 .05-0.2 w-%, and in the case of benzoic acid is in the range between 0.1-0.5 w-%;
 bulk concentration of phenols, triclosan, is in the range between 0.1-0.3 w-%, and
 5 bulk concentration of chlorhexidine is in the range between 0.01-0.05 w-%.

65. The method according to claims 54 to 64,
characterised in that the less soluble amongst the aggregating substances is a
 lipid or lipid-like material, especially a polar lipid, whereas the substance which is
 10 more soluble in the suspending liquid and which lowers the average elastic energy
 of the droplet is a surfactant or else has surfactant-like properties and / or is a form
 of said lipid or lipid-like material which is comparably soluble as said surfactant
 or the surfactant-like material.

66. The method according to claim 65,
characterised in that the lipid or lipid-like material is a lipid or a lipoid from a
 biological source or a corresponding synthetic lipid or any of its modifications,
 said lipid preferably belonging to the class of pure phospholipids corresponding to
 the general formula

20



where R₁ and R₂ is an aliphatic chain, typically a C₁₀₋₂₀-acyl, or -alkyl or partly
 unsaturated fatty acid residue, in particular, an oleoyl-, palmitoeloyl-, elaidoyl-,

linoleyl-, linolenyl-, linolenoyl-, arachidoyl-, vaccinyl-, lauroyl-, myristoyl-,
palmitoyl-, or stearoyl chain; and where R_3 is hydrogen, 2-trimethylamino-1-ethyl,
2-amino-1-ethyl, C_{1-4} -alkyl, C_{1-5} -alkyl substituted with carboxy, C_{2-5} -alkyl
substituted with hydroxy, C_{2-5} -alkyl substituted with carboxy and hydroxy, or C_{2-5} -
5 alkyl substituted with carboxy and amino, inositol, sphingosine, or salts of said
substances, said lipid comprising also glycerides, isoprenoid lipids, steroids,
sterines or sterols, of sulphur- or carbohydrate-containing lipids, or any other
bilayer-forming lipids, in particular half-protonated fluid fatty acids, said lipid is
selected from the group comprising phosphatidylcholines,
10 phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositols,
phosphatidic acids, phosphatidylserines, sphingomyelins or other
sphingophospholipids, glycosphingolipids (including cerebrosides,
ceramidepolyhexosides, sulphatides, sphingoplasmalogens), gangliosides and
other glycolipids or synthetic lipids, in particular with corresponding sphingosine
15 derivatives, or any other glycolipids, whereby two similar or different chains can
be ester-groups-linked to the backbone (as in diacyl and dialkenoyl compound) or
be attached to the backbone with ether bonds, as in dialkyl-lipids.

67. The method according to claim 65,
20 **characterised in that** the surfactant or surfactant-like material preferably is a
nonionic, a zwitterionic, an anionic or a cationic surfactant, especially a fatty-acid
or -alcohol, an alkyl-tri/di/methyl-ammonium salt, an alkylsulphate salt, a
monovalent salt of cholate, deoxycholate, glycocholate, glycodeoxycholate,
taurodeoxycholate, taurocholate, etc., an acyl- or alkanoyl-dimethyl- aminoxide,
25 esp. a dodecyl- dimethyl-aminoxide, an alkyl- or alkanoyl-N-methylglucamide, N-
alkyl-N,N- dimethylglycine, 3-(acyldimethylammonio)-alkanesulphonate, N-acyl-
sulphobetaine, a polyethylene-glycol-octylphenyl ether, esp. a nonaethylene-
glycol-octylphenyl ether, a polyethylene-acyl ether, esp. a nonaethylen-dodecyl
ether, a polyethylene-glycol-isoacyl ether, esp. a octaethylene-glycol-isotridecyl

ether, polyethylene-acyl ether, esp. octaethylenedodecyl ether, polyethylene-glycol-sorbitane-acyl ester, such as polyethyleneglykol-20-monolaurate (Tween 20) or polyethyleneglykol-20-sorbitan-monooleate (Tween 80), a polyhydroxyethylene-acyl ether, esp. polyhydroxyethylene- lauryl, -myristoyl, -cetylstearyl, or -oleoyl ether, as in polyhydroxyethylene-4 or 6 or 8 or 10 or 12, etc., -lauryl ether (as in Brij series), or in the corresponding ester, e.g. of polyhydroxyethylen-8-stearate (Myrj 45), -laurate or -oleate type, or in polyethoxylated castor oil 40, a sorbitane-monoalkylate (e.g. in Arlacel or Span), esp. sorbitane-monolaurate, an acyl- or alkanoyl-N-methylglucamide, esp. in or decanoyl- or dodecanoyl-N-methylglucamide, an alkyl-sulphate (salt), e.g. in lauryl- or oleoyl-sulphate, sodium deoxycholate, sodium glycodeoxycholate, sodium oleate, sodium taurate, a fatty acid salt, such as sodium elaidate, sodium linoleate, sodium laurate, a lysophospholipid, such as n-octadecylene(=oleoyl)-glycerophosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-acyl-, e.g. lauryl or oleoyl-glycerophosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-tetradecyl-glycerophosphatidic acid, -phosphorylglycerol, or -phosphorylserine, a corresponding palmitoeloyl-, elaidoyl-, vaccenyl-lysophospholipid or a corresponding short-chain phospholipid, or else a surface-active polypeptide.

20 68. The method according to claims 54 to 67, **characterised in that** the average diameter of the penetrant is between 30 nm and 500 nm, more preferably between 40 nm and 250 nm, even more preferably between 50 nm and 200 nm and particularly preferably between 60 nm and 150 nm.

25 69. The method according to claims 54 to 68, **characterised in that** the total dry weight of droplets in a formulation is 0.01 weight-% (w-%) to 40 w-% of total formulation mass, more preferably between 0.1 w-% and 30 w-%, and most preferably between 0,5 w-% and 20 w-%.

70. The method according to claims 54 to 69,
characterised in that the total dry weight of droplets in a formulation is selected
to increase the formulation viscosity to maximally 200 mPas, more preferably up
5 to 40 mPas, and most preferably up to 8 mPas.

71. The method according to claims 54 to 70,
characterised in that at least one edge-active substance or surfactant and/or at
least one amphiphilic substance, and / or at least one hydrophilic fluid and the
10 agent are mixed, if required separately, to form a solution, the resulting (partial)
mixtures or solutions are then combined subsequently to induce, preferably by
action of mechanical energy such as shaking, stirring, vibrations, homogenisation,
ultrasonication, shearing, freezing and thawing, or filtration using convenient
driving pressure, the formation of penetrants that associate with and / or
15 incorporate the agent

72. The method according to claim 71,
characterised in that said amphiphilic substances are dissolved in volatile
solvents, such as alcohols, especially ethanol, or in other pharmaceutically
20 acceptable organic solvents, such as ethanol, 1- and 2-propanol, benzyl alcohol,
propylene glycol, polyethylene glycol (molecular weight: 200-400 D) or glycerol,
other pharmaceutically acceptable organic solvents, such as undercooled gas,
especially supercritical CO₂, which are then removed, especially by evaporation or
dilution, prior to making the final preparation.

73. The method according to any one of claims 68 or 72,
characterised in that the formation of said penetrants is induced by the addition
of required substances into a fluid phase, evaporation from a reverse phase, by
injection or dialysis, if necessary under the influence of mechanical stress, such as
25

shaking, stirring, especially high velocity stirring, vibrating, homogenising, ultrasonication, shearing, freezing and thawing, or filtration using a convenient, especially low (1 MPa) or intermediate (up to 10 MPa), driving pressure.

5 74. The method according to claim 73,
characterised in that the formation of said penetrants is induced by filtration, the filtering material having pores sizes between 0.01 μm and 0.8 μm , more preferably between 0.02 μm and 0.3 μm , and most preferably between 0.05 μm and 0.15 μm , whereby several filters may be used sequentially or in parallel.

10

75. The method according to any one of claims 55 to 74,
characterised in that said agents and penetrants are made to associate, at least partly,
– after the formation of said penetrants, e.g. after injecting a solution of the drug
15 in a pharmaceutically acceptable fluid, such as ethanol, 1- and 2-propanol, benzyl alcohol, propylene glycol, polyethylene glycol (molecular weight: 200-400 D) or glycerol into the suspending medium,
– simultaneously with penetrant formation, if required using the drug co-solution and, at least some, penetrant ingredients.

20

76. The method according to any one of the claims 55 to 75,
characterised in that said penetrants, with which the agent is associated, are prepared immediately before the application of the formulation, if convenient, from a suitable concentrate or a lyophilisate.

25

77. The method according to any one of the claims 55 to 76,
characterised in that the formulation is applied by spraying, smearing, rolling or sponging on the application area, in particular by using a metered sprayer, spender, roller or a sponge, or a non-occlusive patch, as appropriate.

78. The method according to any one of the claims 55 to 77,
characterised in that the barrier is skin or at least partly keratinised endothelium
and / or nasal or any other mucosa.

5

79. The method according to claim 78,
characterised in that, the area dose of said penetrant is between 0.1 mg per
square centimetre (mg cm^{-2}) and 40 mg cm^{-2} , more preferably is between
0.25 mg cm^{-2} and 30 mg cm^{-2} and even more preferably is between 0.5 mg cm^{-2}
10 and 15 mg cm^{-2} , in the case that the penetrant is applied on said skin and / or said
at least partly keratinised endothelium.

80. The method according to claim 78,
characterised in that the area dose of said penetrant is between 0.05 mg per
square centimetre (mg cm^{-2}) and 20 mg cm^{-2} , more preferably is between
15 0.1 mg cm^{-2} and 15 mg cm^{-2} and even more preferably is between 0.5 mg cm^{-2} and
10 mg cm^{-2} , in the case that the penetrant is applied on said nasal or other
mucosa.

81. The method according to claim 78,
characterised in that the area dose of said penetrant is between 0.0001 mg per
square centimetre (mg cm^{-2}) and 0.1 mg cm^{-2} , more preferably is between
0.0005 mg cm^{-2} and 0.05 mg cm^{-2} and even more preferably is between 0.001 mg
20 cm^{-2} and 0.01 mg cm^{-2} , in the case that the penetrant is applied on plant body,
25 plant leaves or plant needles.

82. The method of claim 54, used for generating an immune response
on a human or other mammal by vaccinating said mammal.

83. The method of claim 54, used for generating a therapeutic effect in a human or other mammal.

84. The method of claim 54 for the treatment of inflammatory disease,
5 dermatosis, kidney or liver failure, adrenal insufficiency, aspiration syndrome, Behcet syndrome, bites and stings, blood disorders, such as cold-haemagglutinin disease, haemolytic anemia, hypereosinophilia, hypoplastic anemia, macroglobulinaemia, trombocytopenic purpura, furthermore, for the management of bone disorders, cerebral oedema, Cogan's syndrome, congenital adrenal
10 hyperplasia, connective tissue disorders, such as lichen, lupus erythematosus, polymyalgia rheumatica, polymyositis and dermatomyositis, epilepsy, eye disorders, such as cataracts, Graves' ophthalmopathy, haemangioma, herpes infections, neuropathies, retinal vasculitis, scleritis, for some gastro-intestinal disorders, such as inflammatory bowel disease, nausea and oesophageal damage,
15 for hypercalcaemia, infections, e.g. of the eye (as in infections mononucleosis), for Kawasaki disease, myasthenia gravis, various pain syndromes, such as postherpetic neuralgia, for polyneuropathies, pancreatitis, in respiratory disorders, such as asthma, for the management of rheumatoid disease and osteoarthritis, rhinitis, sarcoidosis, skin diseases, such as alopecia, eczema, erythema
20 multiforme, lichen, pemphigus and pemphigoid, psoriasis, pyoderma gangrenosum, urticaria, in case of thyroid and vascular disorders.

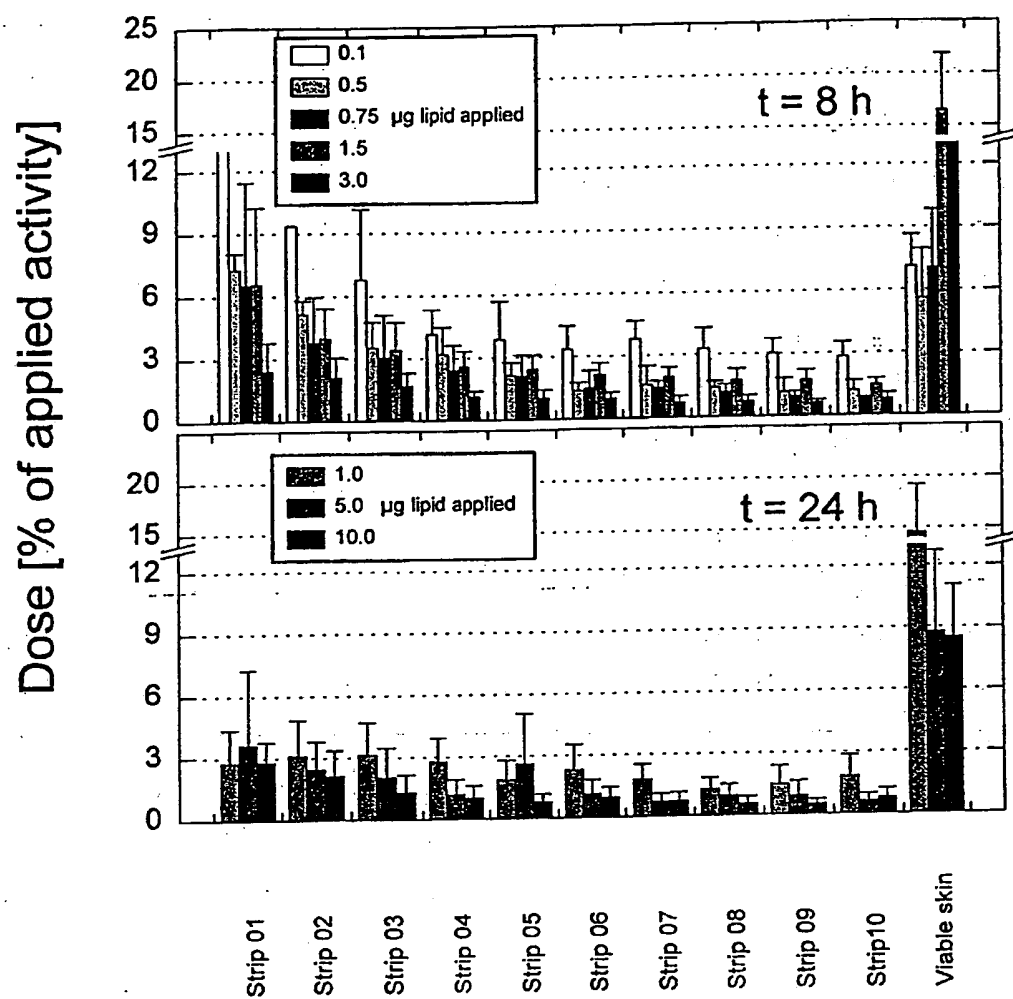


Figure 1

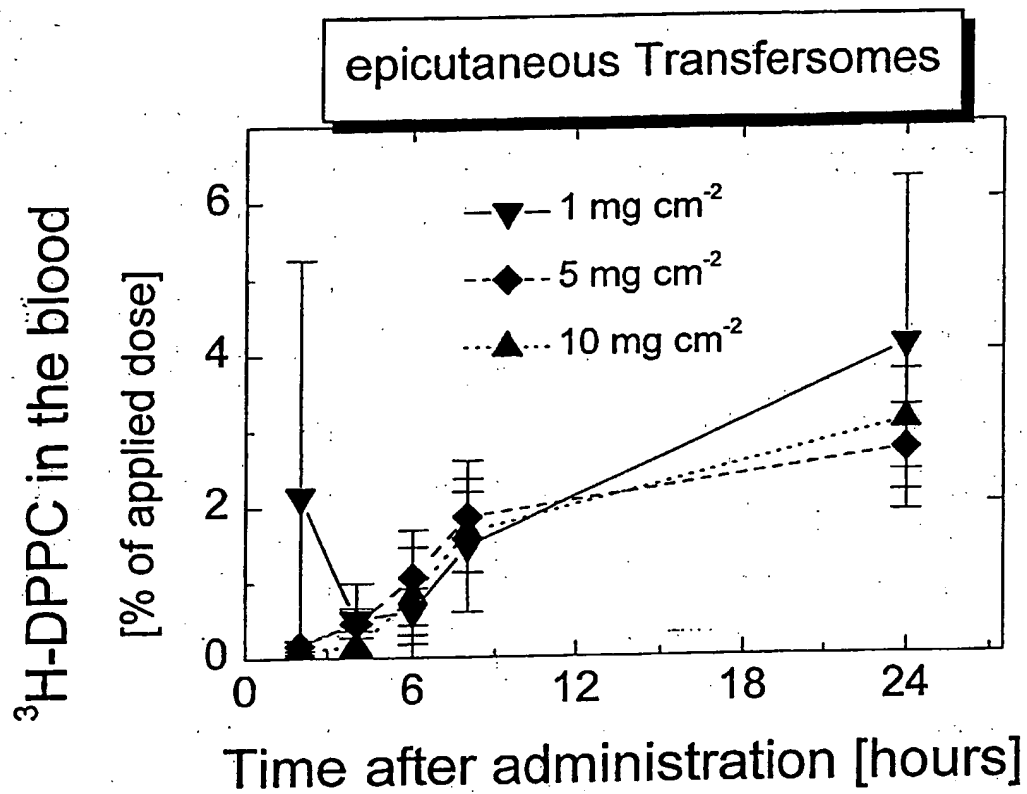


Figure 2

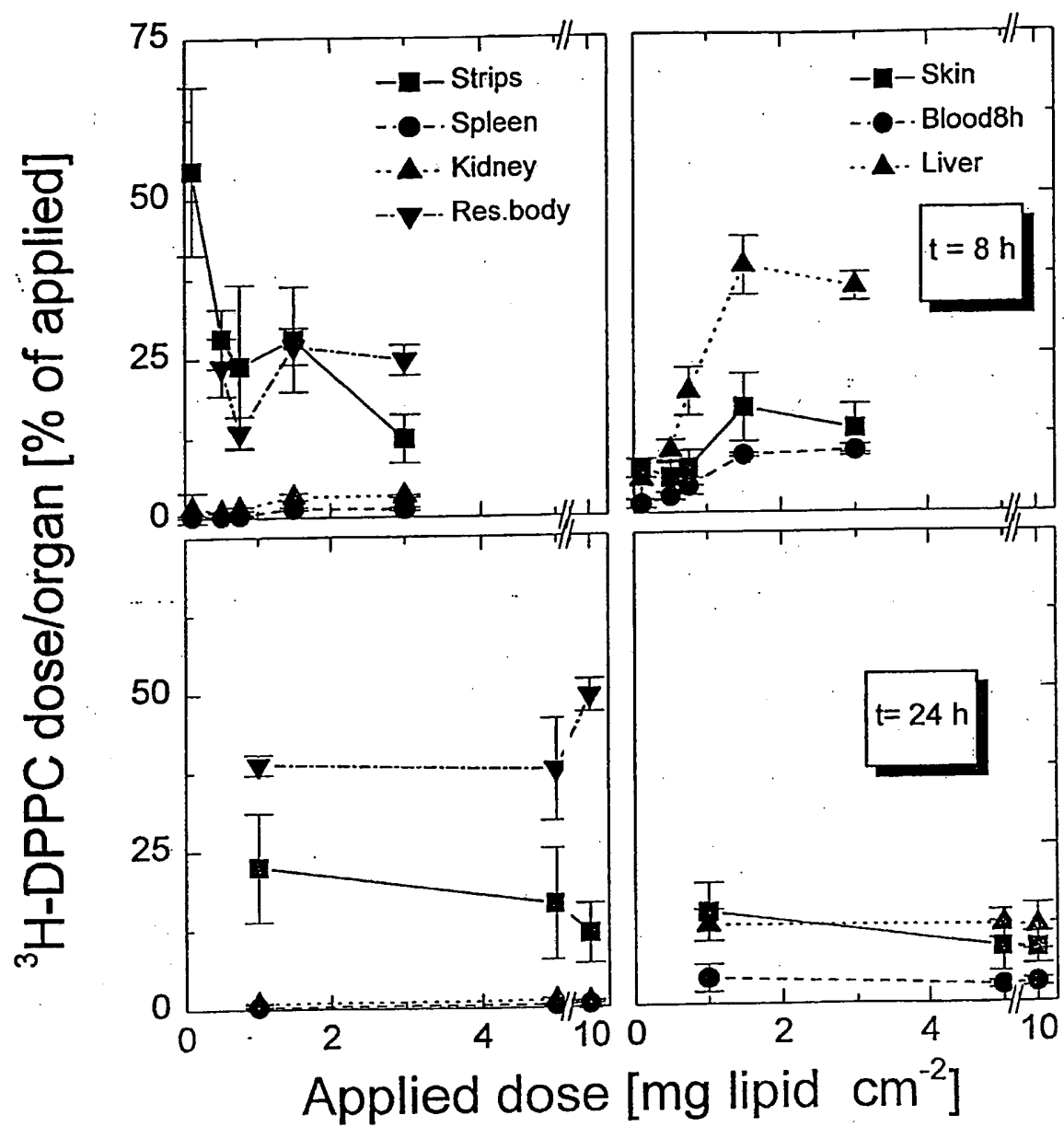


Figure 3

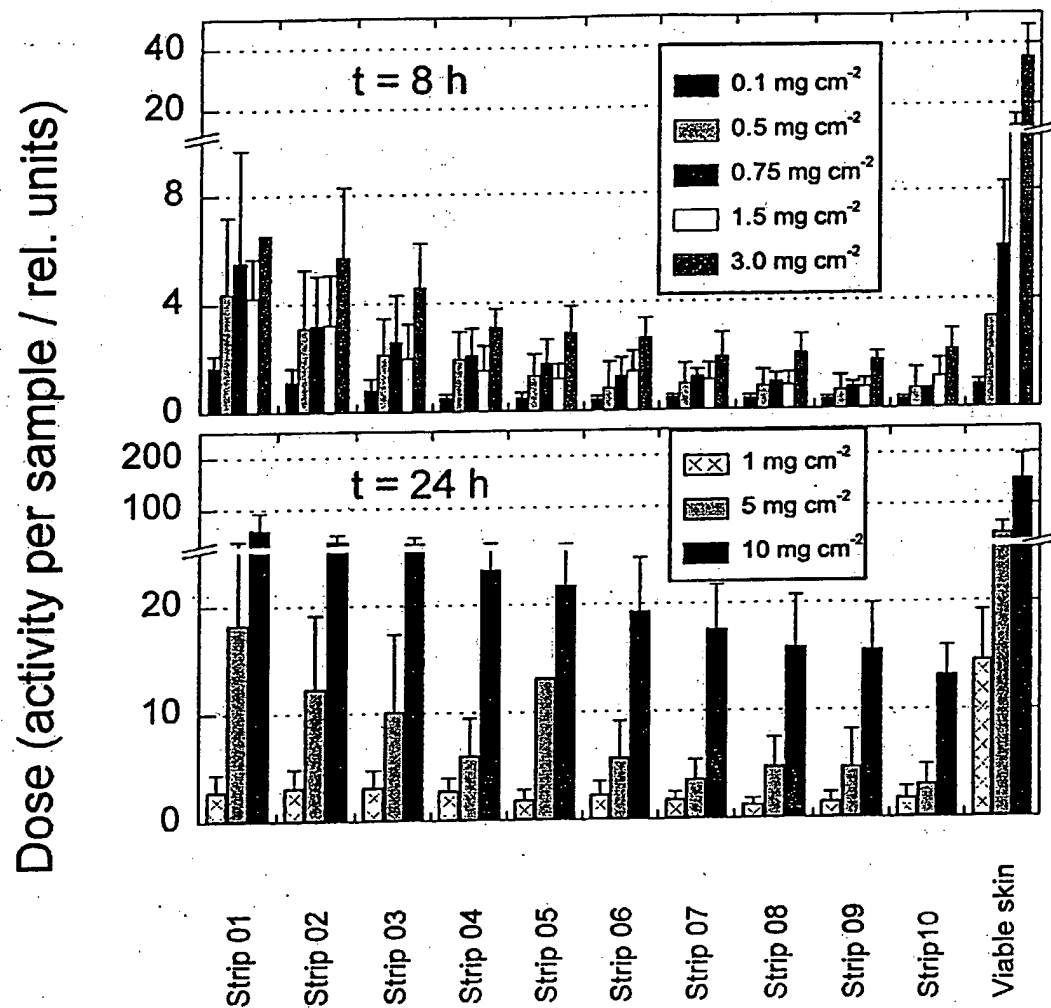


Figure 4

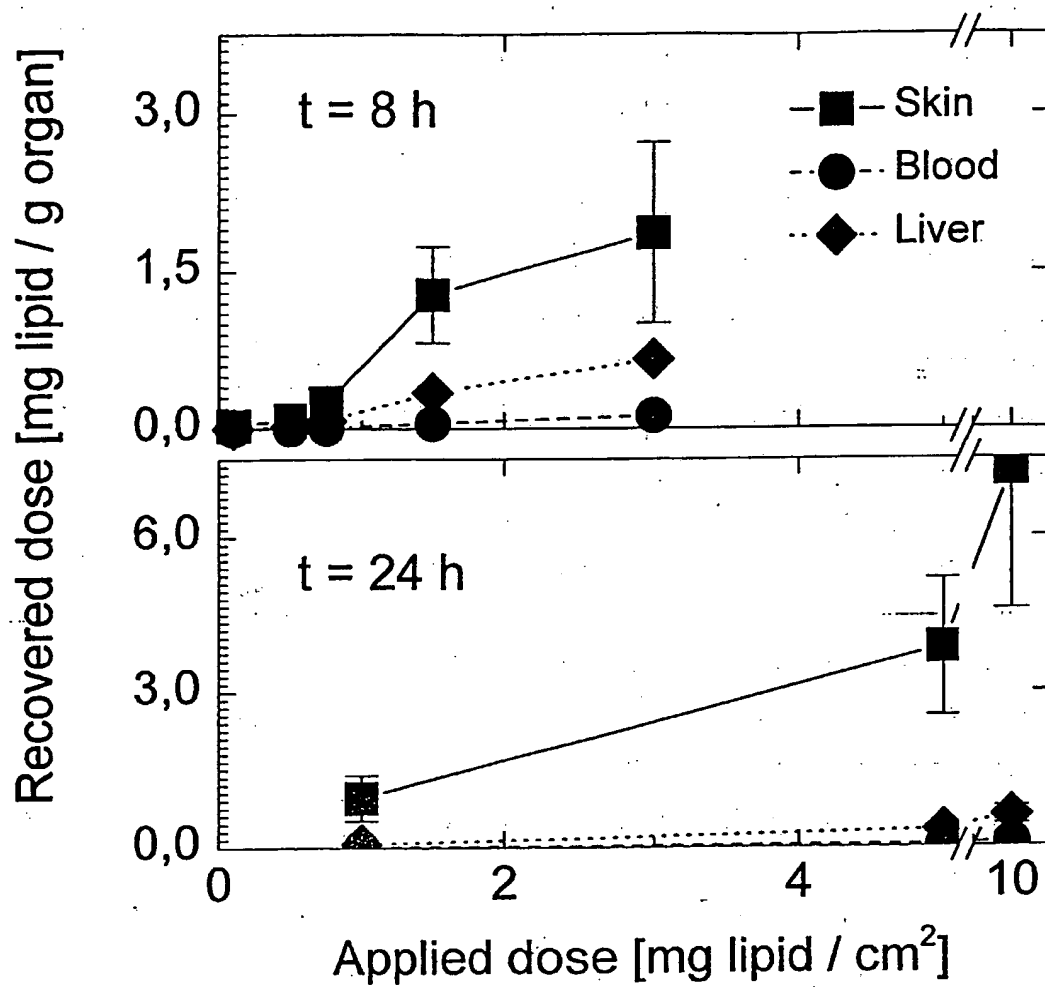


Figure 5

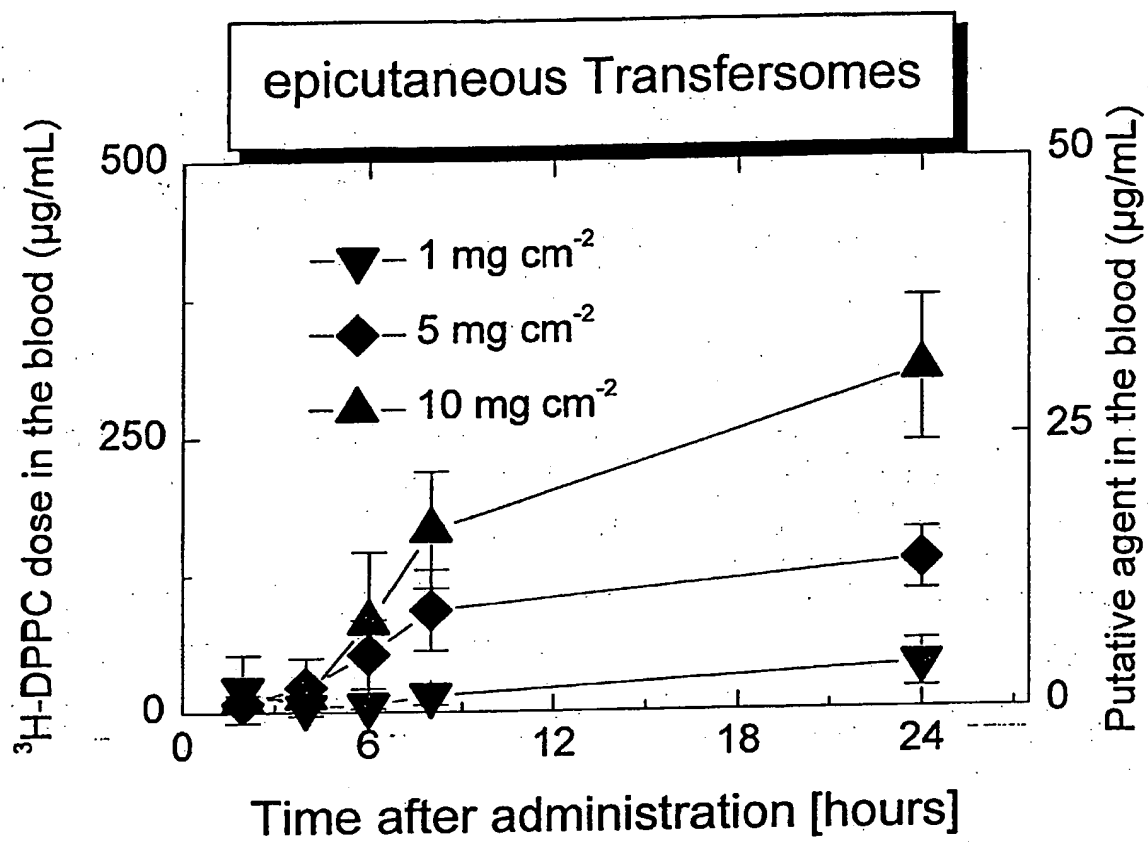


Figure 6

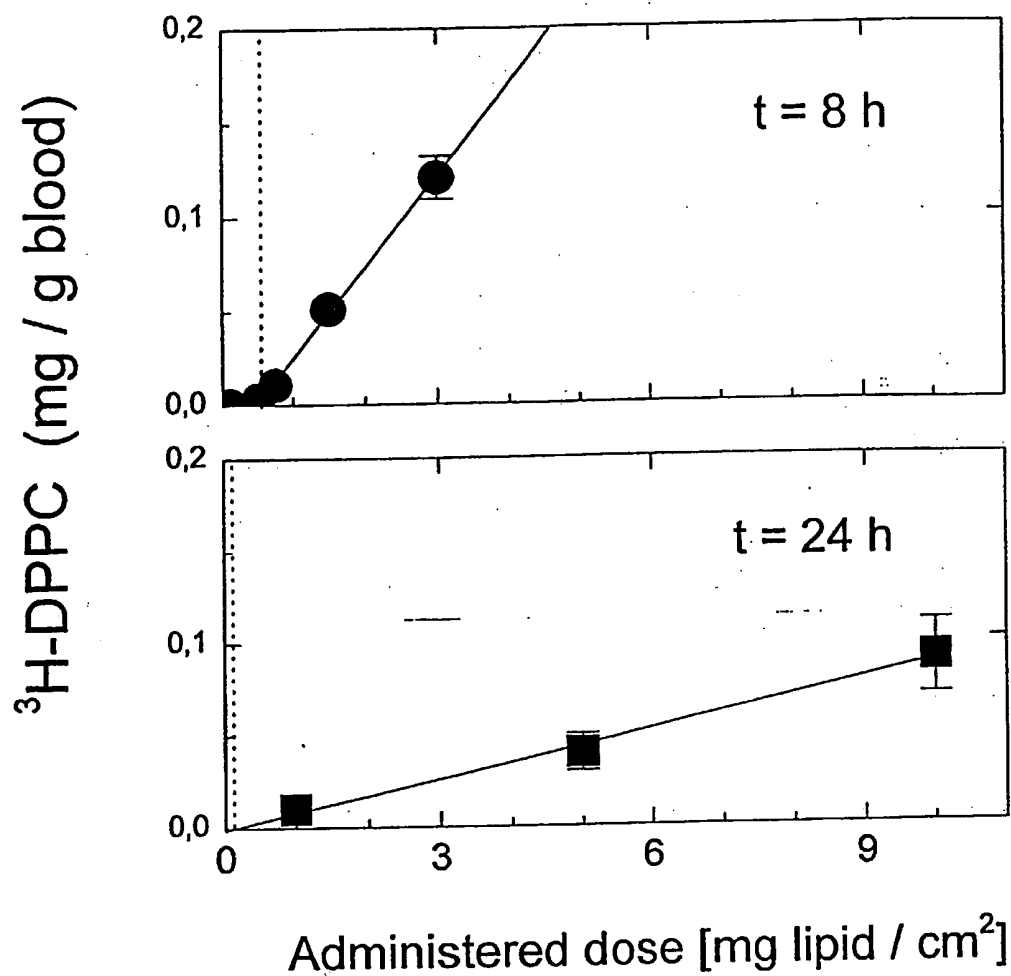
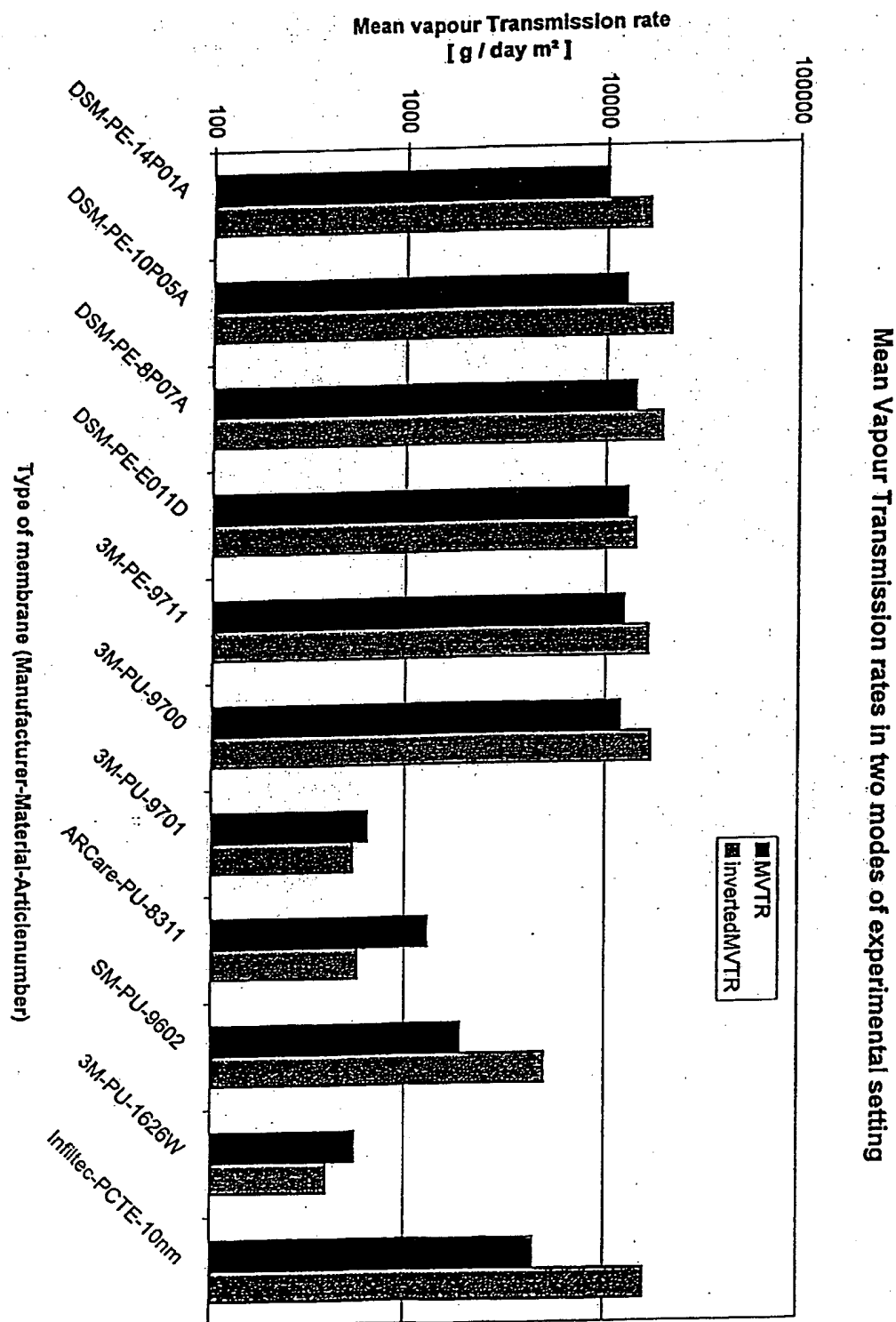


Figure 7

Figure 8



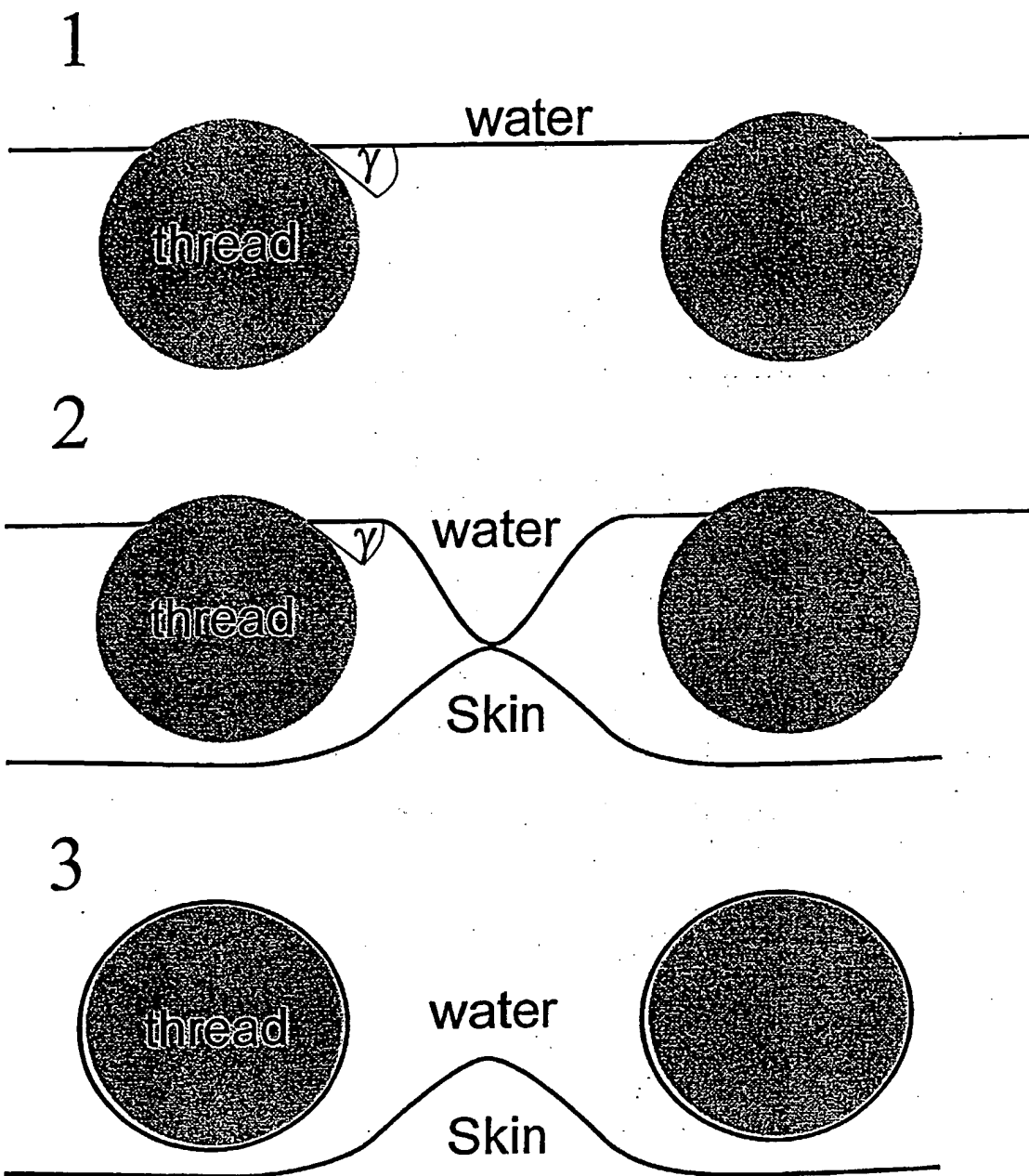
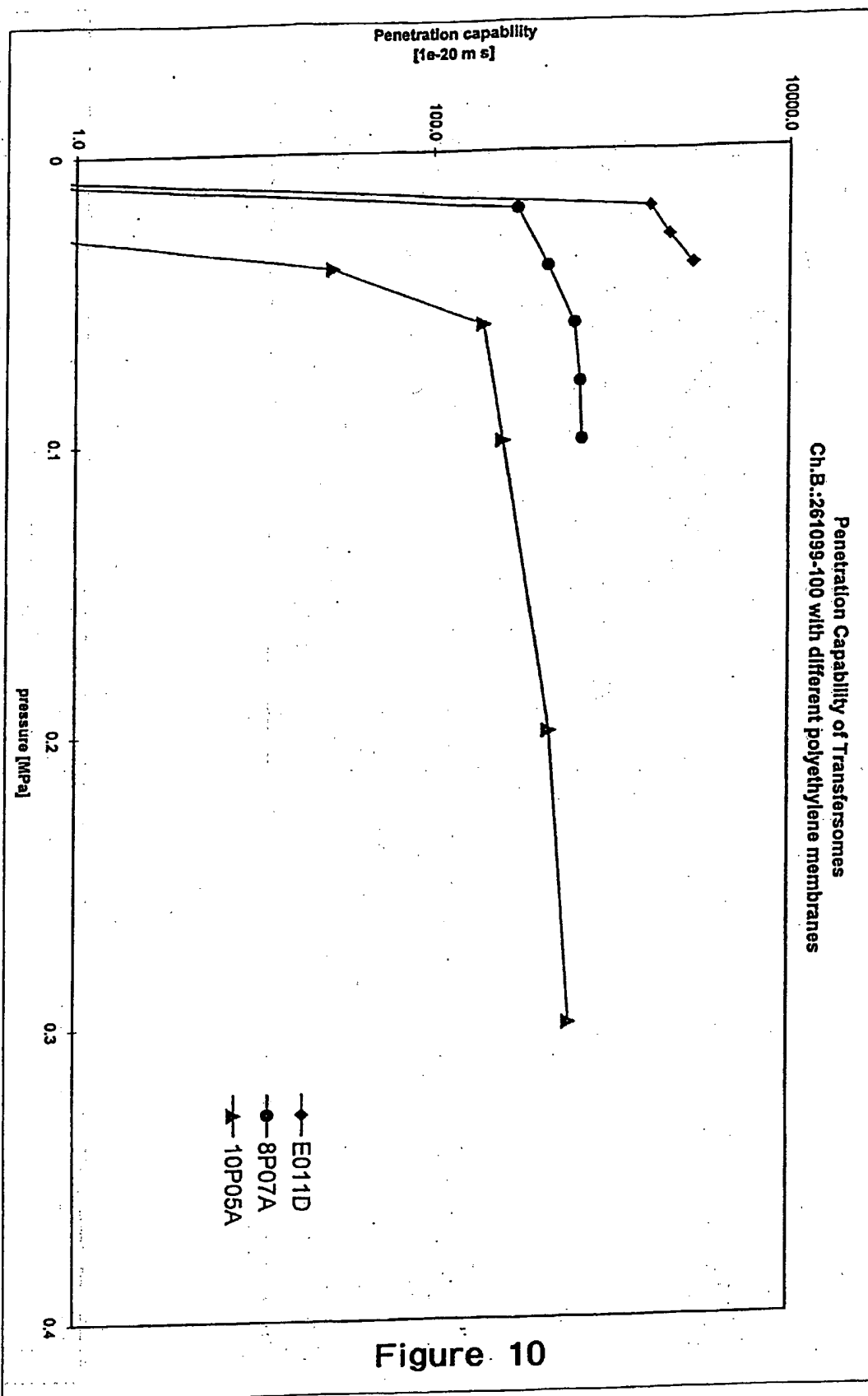


Figure 9



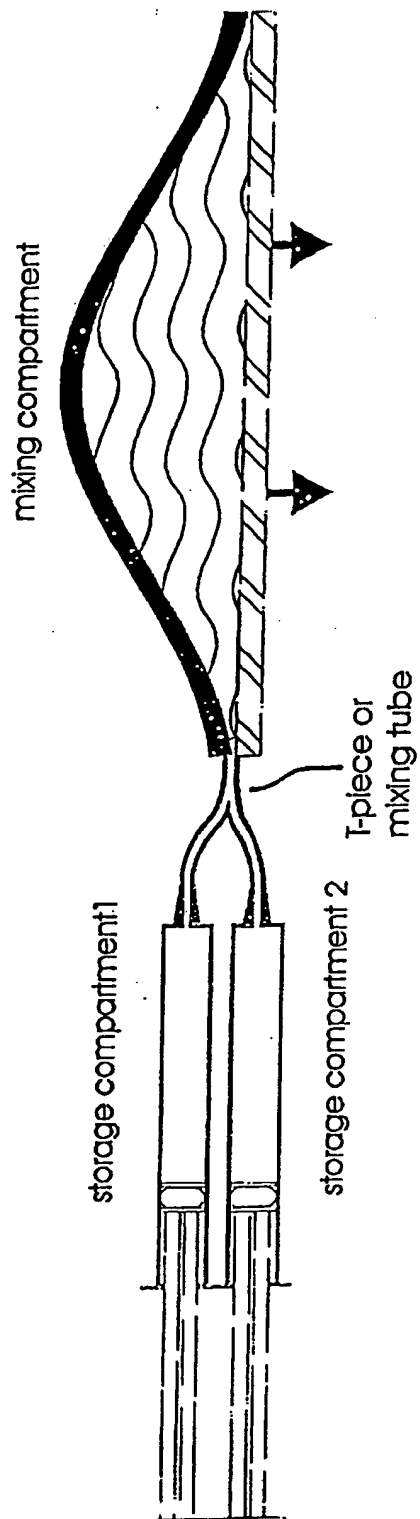


Figure 11

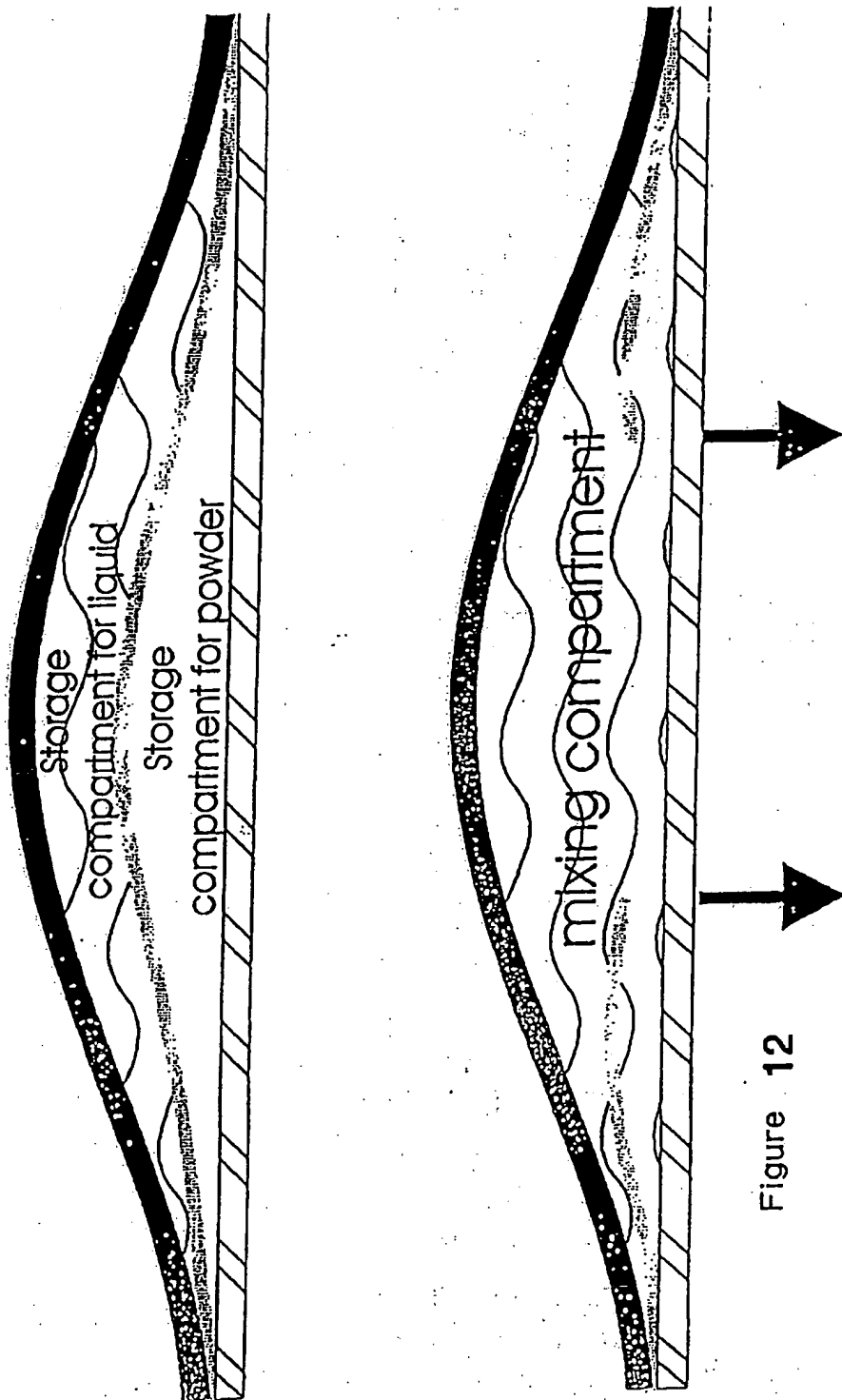


Figure 12

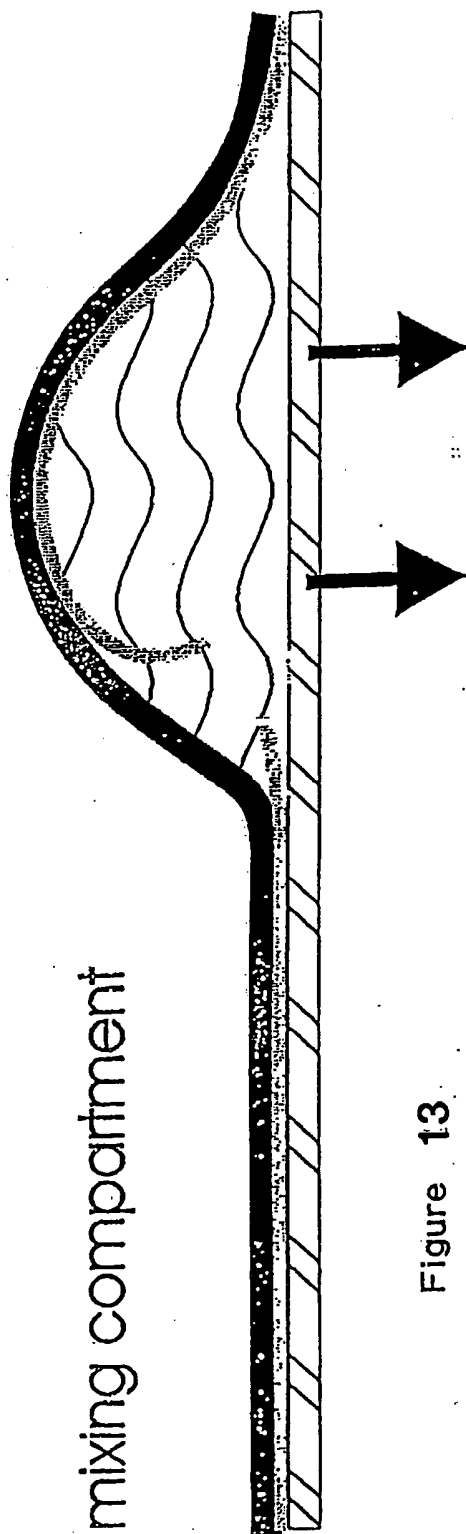
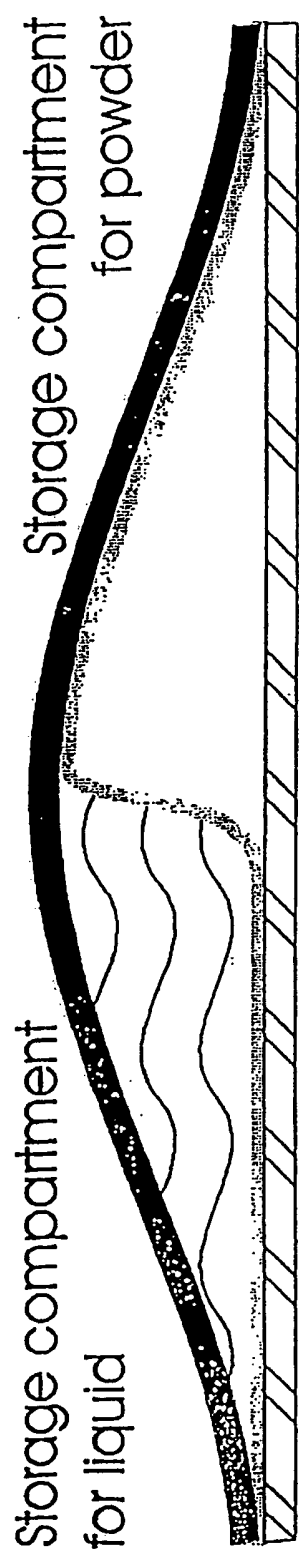
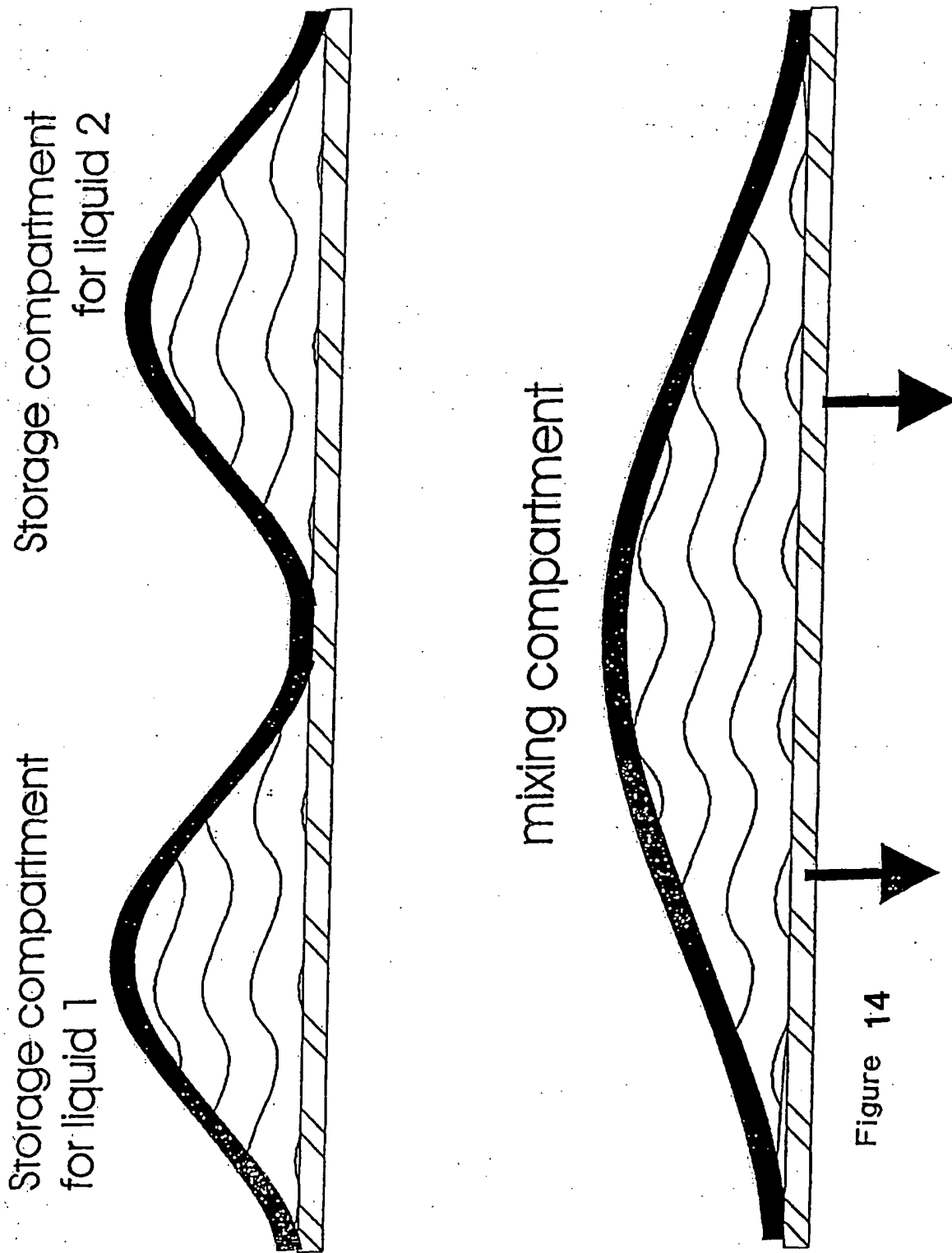


Figure 13



INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/06367

A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

WPI Data, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>G. CEVC ET AL.: "transfersomes-mediated transepidermal delivery improves the regiospecificity and biological activity of corticosteroids in vivo" JOURNAL OF CONTROLLED RELEASE, vol. 45, no. 3, 7 April 1997 (1997-04-07), pages 211-226, XP000640528 Amsterdam (NL) page 211, abstract page 225, conclusions page 213, paragraph 2.1.</p> <p style="text-align: center;">--- -/-</p>	<p>1,2, 12-15, 18-29, 54,55, 65-75, 78-81, 83,84</p>

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

*** Special categories of cited documents:**

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T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 98 17255 A (CEVC) 30 April 1998 (1998-04-30)</p> <p>the whole document page 19, line 21 - line 25 page 21, line 16 -page 23, line 17 page 24, line 9 - line 25 page 27, line 4 -page 28, line 4 claim 18</p>	<p>1-28, 54-57, 61,63, 65-69, 71-76, 78-84</p>
A	<p>V.M. KNEPP ET AL.: "controlled drug release from a novel liposomal delivery system. II. transdermal delivery characteristics" JOURNAL OF CONTROLLED RELEASE, vol. 12, no. 1, March 1990 (1990-03), pages 25-30, XP000113393 Amsterdam (NL) page 26, column 1, paragraph 6. page 26, column 2, paragraph 2</p>	29-32
A	<p>EP 0 674 913 A (LECTEC CORPORATION) 4 October 1995 (1995-10-04) the abstract</p>	1,29-35
A	<p>WO 98 30215 A (CILAG) 16 July 1998 (1998-07-16) claims 1-9</p>	1,4-11

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 00/06367

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9817255 A	30-04-1998	AU 724218 B	14-09-2000
		AU 4510897 A	15-05-1998
		BR 9612750 A	28-12-1999
		EP 0935457 A	18-08-1999
		HU 9903363 A	28-03-2000
EP 674913 A	04-10-1995	US 5536263 A	16-07-1996
		AU 676623 B	13-03-1997
		AU 1002495 A	12-10-1995
		CA 2133598 A	01-10-1995
		FI 950465 A	01-10-1995
		JP 7265353 A	17-10-1995
		NO 951217 A	02-10-1995
		US 6096334 A	01-08-2000
		US 5741510 A	21-04-1998
WO 9830215 A	16-07-1998	US 6096333 A	01-08-2000
		AU 5777498 A	03-08-1998

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(74) Agent: MAIWALD, Walter; Maiwald Patentan-
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HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,
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ning of each regular issue of the PCT Gazette.

(54) Title: A METHOD FOR THE IMPROVEMENT OF TRANSPORT ACROSS ADAPTABLE SEMI-PERMEABLE BARRI-
ERS

(57) Abstract: The invention relates to a method, a kit and a device for controlling the flux of penetrants across an adaptable semi-permeable porous barrier, the method comprising the steps of: preparing a formulation by suspending or dispersing said penetrants in a polar liquid in the form of fluid droplets surrounded by a membrane-like coating of one or several layers, said coating comprising at least two kinds of forms of amphiphilic substances with a tendency to aggregate, said penetrants being able to transport agents through the pores of said barrier or to enable agent permeation through the pores of said barrier after penetrants have entered the pores, selecting a dose amount of said penetrants to be applied on a predetermined area of said barrier to control the flux of said penetrants across said barrier, and applying the selected dose amount of said formulation containing said penetrants onto said area of said porous barrier.

WO 01/01963 A1

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(51) Cl.Int.⁵/Int.Cl.⁵ A61K 9/127
(72) Inventeur/Inventor:
Cevc, Gregor, DE
(73) Propriétaire/Owner:
IDEA AG, DE
(74) Agent: KIRBY EADES GALE BAKER

(54) Titre : PREPARATION VISANT L'APPLICATION D'AGENTS SOUS FORME DE MINI-GOUTTELETTES
(54) Title: PREPARATION FOR THE APPLICATION OF AGENTS IN MINI-DROPLETS

(57) Abrégé/Abstract:

The invention relates to a preparation for the application of agents in the form of minuscule droplets of fluid, in particular provided with membrane-like structures consisting of one or several layers of amphiphilic molecules, or an amphiphilic carrier substance, in particular for transporting the agent into and through natural barriers such as skin and similar materials. The preparation contains a concentration of edge active substances which amounts to up to 99 mol-% of the agent concentration which is required for the induction of droplet solubilization. Such preparations are suitable, for example, for the non-invasive applications of antidiabetics, in particular of insulin. The invention, moreover, relates to the methods for the preparation of such formulations,

Abstract

The invention relates to a preparation for the application of agents in the form of minuscule droplets of fluid, in particular provided with membrane-like structures consisting of one or several layers of amphiphilic molecules, or an amphiphilic carrier substance, in particular for transporting the agent into and through natural barriers such as skin and similar materials. The preparation contains a concentration of edge active substances which amounts to up to 99 mol-% of the agent concentration which is required for the induction of droplet solubilization. Such preparations are suitable, for example, for the non-invasive applications of antidiabetics, in particular of insulin. The invention, moreover, relates to the methods for the preparation of such-formulations.

International patent application

Preparation for the application of agents in mini-droplets

The present invention relates to a novel type of preparations suitable for the application of different agents in the form of a minuscule droplet or, in particular, a vesicle consisting of one or a few membrane-like amphiphile assemblies. These can mediate the transport of agents into and through a series of natural permeability barriers or through the constrictions in such barriers; for example, through intact skin or similar organs. The invention further relates to a procedure for the large-scale production of such carriers. As a special example, non-invasive application of antidiabetics is described for the case of insulin.

The application of various agents is often hampered by the presence of barriers with a low permeability to such agents. Owing to skin impermeability, for example, many common therapeutic agents must be applied per os or parenterally (i.v., i.m., i.p.). Intrapulmonary and intranasal applications of aerosols, the use of rectal formulations, gels for mucous applications, or use of ocular formulations are only practicable in certain areas and not for all types of drugs. The transport of different agents into plant tissues is subject to even more severe constraints due to the high permeability barrier of the cuticular wax layers.

Noninvasive drug application through permeability barriers thus would be advantageous in many cases. In humans and animals one would expect such a percutaneous application of agents to protect the agents from degradation in the gastrointestinal tract; modified drug distribution could possibly also be achieved. Such drug application, moreover, would influence the pharmacokinetics of the agent molecules and permit simple as well as multiple noninvasive therapy. (Karzel K., Liedtke, R.K. (1989) *Arzneim. Forsch./Drug Res.* 39, 1487-1491). In the case of plants, improved penetration

into or through the cuticle could reduce the drug concentration required for a given application and thus significantly diminish pollution problems (Price, C.E. (1981) In: The plant cuticle (D.F. Cutler, K.L. Alvin, C.E. Price, Edits.), Academic, New York, pp. 237-252).

There are many reports on different attempts to increase the permeability of intact skin by suitable manipulations (cf. Karzel und Liedtke, op. cit.). Jet injection (Siddiqui & Chien (1987) Crit. Rev. Ther. Drug. Carrier. Syst. 3, 195-208.), the use of electric fields (Burnette & Ongpipattanakul (1987) J. Pharm. Sci. 76, 765-773) or chemical penetration enhancers, such as solvents and surfactants, are particularly worth mentioning. A long list of additives which have been used to enhance the penetration of one particular water soluble agent (Nolaxon) into skin, for example, is given in the work by Aungst et al. (1986, Int. J. Pharm. 33, 225-234). This list encompasses nonionic substances (including long-chain alcohols, surfactants, zwitterionic phospholipids, etc.), anionics (most notably fatty acids), cationic long-chain amines, sulfoxides as well as different amino-derivatives; amphotheric glycines and betaines are also mentioned. Despite all this, the problem of agent penetration into skin has as yet not at all - or not satisfactorily - been solved.

A survey of procedures used for increasing the penetration of agents through a plant cuticle is given in the work by Price (1981, op.cit.). To date it has been common to simply add chemical penetration enhancers to the mixture of agent and other molecules; applications to human skin were the only case in which additives were sometimes applied in advance, in the form of an organic solution. The reason for this application form was the current concept for the action of penetration enhancers: to date one has studied, discussed, and believed

that, in general, any facilitated agent penetration is a consequence of skin fluidization, on the one hand (Golden et al., (1987) J. Pharm. Sci. 76, 25-28). (This phenomenon is normally associated with a destruction of the skin surface and of its protective shield and thus is undesired.) On the other hand, it has been shown that some agents can permeate through skin in the form of low-molecular weight complexes with added molecules (Green et al., (1988) Int. J. Pharm. 48, 103-111).

Methods deviating from the ones already described have brought little improvement to date. The use of lipoidal carriers, the liposomes, on intact skin, which has been theoretically discussed by several authors, was mainly aimed at modifying the agent's pharmacokinetics (Patel, Bioch. Soc. Trans., 609th Meeting, 13, 513-517, 1985, Mezei, M. Top. Pharm. Sci. (Proc. 45th Int. Congr. Pharm. Sci.F.I.P.) 345-58 Elsevier, Amsterdam, 1985). Thus far, all proposal of this kind, moreover, involved the use of standard lipid vesicles (liposomes) which cannot penetrate the skin at all or permeate through the skin very inefficiently, as is shown in this patent application. Patent applications nos. JP 61/271204 A2 [86/271204] refer to a related use of liposomes in which hydrochinonglucosidal is employed to improve the stability of the agent.

Hitherto available preparations for percutaneous use have mostly been applied under occlusion; in the case of liposomal preparations, this was even a general rule. The corresponding preparations only contained small or lipophilic substances, as well as a limited number of skin-fluidizing additives. Correspondingly, they afforded only partial control over the pharmacokinetic properties of final preparations. In an attempt to improve this situation a proposal was made (WO 87/1938 A1) to use drug-carrying lipid vesicles in combination with a gelatinizing agent as a transdermal patch. This has

prolonged drug action but has not increased the skin-penetration capability of the drug itself. Through massive use of penetration enhancers (polyethylene glycol and fatty acids) and of lipid vesicles, Gesztes and Mezei (1988, Anesth. Analg. 67, 1079-1081) have succeeded in inducing local analgesia with lidocaine-containing carriers; however, the overall effectiveness of the drug in this preparation was relatively low and its effects were only observed several hours after the beginning of an occlusive application.

By a specially designed formulation we have succeeded in obtaining results which were dramatically better than those of Gesztes and Mezei. Our carrier formulations consisted of filtered lipid vesicles (liposomes) which also contained some detergents, with a declared optimum lipid/surfactant content of 1-40/1, in practice mainly around 4/1.

These results provided a basis for German patent application P 40 26 834.9-41 which also refers to German patent application P 40 26 833.0-43; the latter deals with the problem of liposome fabrication.

Since then, we have unexpectedly discovered that certain criteria, described in this application, may be formulated for the qualification of drug carriers as suitable for the penetration into and through a permeability barrier. The main requirement of such a drug carrier - which in the following is called a transfersome - is that it is sufficiently elastic to penetrate through the constrictions in a barrier, such as skin. In the case of transfersomes consisting of phosphatidylcholine and sodium cholate this condition is fulfilled when the surface-tension of a carrier is below 10 Piconewton; similar values are also likely to pertain to other, related systems. Carriers which are capable of creating a gradient after an application are particularly

useful; this is due to the fact that they have a spontaneous tendency for penetration through permeability barriers.

It is, therefore, an object of the present invention to specify the properties of novel preparations which are suitable for the mediation of rapid transport of diverse agents and other substances through permeability barriers and constrictions.

A further object of this invention is to introduce a new class of carrier preparations for the transport of drugs through human, animal or plant skin, which result in a characteristic improved availability of the agent molecules at the target site.

It is yet another object of this invention to prepare formulations for non-invasive application of antidiabetics, most notably of insulin; these should ensure an improved, therapeutically sufficient, and reproducible form of drug application.

A further object of this invention is to provide procedures for the production of such preparations.

These objects have been accomplished through the features of the independent claims.

Advantageous embodiments are mentioned in the subclaims.

Brief Description of the Drawings

Figure 1 is a graph showing the permeation resistance (left plot) and the size distribution (right plot) of vesicles according to Examples 1 to 13 (o) and Examples 14 to 20 (+).

Figure 2 shows the size of vesicles according to Examples 21 to 31.

Figure 3 is a graph showing the permeation resistance (left plot) and the size distribution (right plot) of vesicles according to Examples 32 to 39. The sizes have been measured after permeation.

Figure 4 shows the vesicle size distribution of Examples 32 to 39 after 2 and 40 days.

Figure 5 shows the permeation resistance (left plot) and the size distribution after permeation (right plot) of vesicles according to Examples 40 to 49 (broad lines in left plot) and of Examples 50 to 61 (thin line in left plot).

Figure 6 shows graphs of the permeation resistance (left plot) and size distribution (right plot) of vesicles according to Examples 62 to 75.

Figure 7 is a graph showing characteristics and deformability of vesicles in a bilayer analysis of the data points of Examples 99 to 107.

Figure 8 is a graph showing the permeation resistance (left plot) and size distribution (right plot) of vesicles according to Examples 108 to 119.

Figure 9 is a graph showing the permeation resistance (left plot) and size distribution (right plot) of vesicles according to Examples 129 to 136.

Figure 10 is a graph showing the percutaneous absorption of inventive transfersomes by skin according to Examples 151 to 157.

Figure 11 is a graph showing the dosage of systematically administered transfersomes in blood according to Examples 158 to 162.

Figure 12 shows the concentration of tritium insulin in blood, which was administered percutaneously via inventive transfersomes, in comparison to conventional liposomes according to Examples 163 to 165.

Figure 13 shows the effect of percutaneously administered insulin on the glucose concentration in blood according to Example 166.

Figure 14 is a graph showing the permeation resistance (left plot) and the size distribution (right plot) of vesicles according to Example 201 to 215.

Figure 15 is a graph showing the permeation resistance (left plot) and size distribution (right plot) of vesicles according to Examples 216 to 235.

Figure 16 is a graph showing the outer diameter in relation to the Tween 80 content of the vesicles between solubilization and vesicularization according to Examples 173 to 175.

Figure 17 shows the glucose uptake in blood after insulin administration with liposomes containing surface active agents according to Example 236.

Figure 18 is a graph showing the glucose uptake in blood after insulin administration via transfersomes according to Example 237.

Figure 19 is a graph showing the glucose uptake in blood after insulin administration via transfersomes according to Example 238.

Figure 20 shows the results of three percutaneous applications of insulin with transfersomes compared with two subcutaneous injections of insulin according to Example 238.

Figure 21 is a graph showing the glucose uptake in blood after administration of insulin via transfersomes according to Example 243.

The transfersomes according to this invention differ from the liposomes hitherto described for topical application and from other related carriers in at least three basic features.

Firstly, they can consist of an arbitrary amphiphile, including oils. Secondly, they can be made in arbitrary fashion: their penetration capacity does not depend on the

manufacturing procedure. Thirdly, the penetration capability of the previously described liposomes optimized for application on skin (cf. patent application P 40 26 834.9-41) was based on the use of a carrier composition with an optimal lipid/surfactant ratio in the range of $L/S=1-40/1$. However, a transfersome must mainly have an optimal elasticity, which ensures a sufficiently high permeation capability of such a carrier. If this basic requirement is fulfilled by the addition of surface-active substances to a basic transfersome component, the necessary total amount of the surface-active substance can correspond to L/S values below $1/500$ (in the case of classical surfactants below $1/50$ to $1/100$). The range of concentrations suitable for making transfersomes is thus by several thousand percent higher than previously believed.

Transfersomes also differ from micellar carrier formulations in at least two basic features. Firstly, a transfersome is, as a rule, far bigger than a micelle; consequently, it also obeys different diffusion laws. Secondly, and more importantly, a transfersome typically contains a water-filled central core (the inner lumen of a vesicle). Nearly all water soluble substances can be incorporated in the core of a transfersome and thus transported across a permeability barrier. Transfersomes are suitable for transporting amphiphilic and lipophilic substances.

If simple carriers are not sufficiently deformable and their permeation capacity must be achieved by using certain surface active additives, the concentration of the latter is then preferably in the range between 0.1 and 99% of the quantity which would be required for carrier solubilization.

Frequently, the optimum - depending on the purpose and the drug used - is located in the range between 1 and 80%, most frequently between 10 and 60% of the solubilization dose; the

concentration range between 20 and 50 mol-% is the most preferred dose.

Our novel transfersomes can mediate transport of agents through essentially all permeability barriers and are suitable, for example, for percutaneous (dermal) applications of medical agents. Transfersomes can carry water- or fat-soluble agents to various depths at the application site, depending on the transfersomal composition, application dose, and form. Special properties which cause a carrier to behave as a transfersome can be realized for phospholipid vesicles as well as for other types of amphiphile aggregates.

In this application it is shown for the first time that by means of suitably formulated transfersomes, a major proportion of the drugs applied can be introduced not only into a permeability barrier, such as skin, but, moreover, can be transported into the deeper tissues where they become systemically active. Transfersomes can carry polypeptides, for example, through intact skin at an effectiveness which is a 1,000 times higher than was previously possible when using structureless penetration enhancers. Transfersomally formulated substances can reach nearly 100 % of the corresponding biological or therapeutical maximum efficacy after applications on human skin. Similar effects, to date, have only been achievable by using an injection needle.

In the course of this study, it has surprisingly been found that through use of such novel drug carriers, antidiabetics can be brought into the blood through intact skin without the necessity of auxiliary measures such as an injection. After a dermal application of insulin applied in the form of transfersomes, more than 50 % and often more than 90 % of the applied drug dose are routinely found in the destined organs of the body. Insulin-containing, dermally applied

transfersomes can thus successfully replace injections of insulin solutions.

The present invention, consequently, opens up a way for simple, noninvasive and completely painless therapy of type II diabetes: transfersomes can be used alone or in combination with an arbitrary dosing means for non-problematic therapy of acute and/or chronic diabetes.

Carriers according to this invention can consist of one or several components. Most commonly, a mixture of basic substances, one or several surface-active substances and agents is used. Lipids and other amphiphiles are best suited basic substances; surfactants or suitable solvents are the best choice from the point of view of surface-active substances. All these can be mixed with agents in certain proportions depending both on the choice of the starting substances and on their absolute concentrations. It is possible that one or several preparation components are only made surface-active by subsequent chemical or biochemical modification of a preparation (ex tempore and/or in situ).

Transfersomes thus offer an elegant, uniform and generally useful means of transport across permeability barriers for diverse agents. These newly developed carriers are perfectly suited for use in human and animal medicine, dermatology, cosmetics, biology, biotechnology, agrotechnology and other fields.

A transfersome according to this invention comprises any carrier with a special capability to get or diffuse into or through a permeability barrier under the effect of a gradient and by so doing to transport material between the application and destination sites.

A (drug) carrier of this type preferably corresponds to a molecular homo- or hetero-aggregate or to a polymer. The carrier aggregate, according to this invention, consists of a few or many, identical or different molecules; these form a physico-chemical, physical, thermodynamical and, quite frequently, functional unity. Some examples of corresponding aggregates are micelles, disk-micelles, oil-droplets (nanoemulsions), nanoparticles, vesicles or 'particulate emulsions'; parts of an aggregate can also be held together by (a) non-covalent force(s). The optimal carrier size is also a function of the barrier properties. Furthermore, it is influenced by the polarity (hydrophilicity), mobility (dynamics), and charge density as well as the elasticity of an carrier (surface). Advantageous sizes of transfersomes are in the range of 10 nm to 10,000 nm.

For dermal applications, for example, preferably particles or vesicles with a diameter of the order of 100-10,000 nm, frequently in the range of 100 to 400 nm, and most frequently with sizes between 100 and 200 nm are used as carriers.

For the use in plants, relatively small carriers, depending on the details of each individual application, should be used, most frequently with diameters below 500 nm.

DEFINITIONS

LIPIDS

A lipid in the sense of this invention is any substance with characteristics similar to those of fats or fatty materials. As a rule, molecules of this type possess an extended apolar

region (chain, X) and, in the majority of cases, also a water-soluble, polar, hydrophilic group, the so-called head-group (Y). The basic structural formula 1 for such substances reads



where n is greater or equal zero. Lipids with n=0 are called apolar lipids; those with n ≥ 1 are polar lipids. In this context, all amphiphiles, such as glycerides, glycerophospholipids, glycerophosphinolipids, glycerophosphonolipids, sulfolipids, sphingolipids, isoprenoidlipids, steroids, sterines or sterols and lipids containing carbohydrate residues, can simply be referred to as lipids.

A phospholipid, for example, is any compound of formula 2



In this formula, n and R₄ have the same significance as in formula 8 except that R₁ and R₂ cannot be hydrogen, an OH-group or a short chain alkyl residue; R₃ is a hydrogen atom or an OH-group, in the majority of cases. In addition, R₄ can be a short chain alkyl group substituted by three short chain alkylammonium residues, e.g. trimethylammonium, or an amino-substituted short chain alkyl, e.g. 2-trimethylammonioethyl (cholinyl).

A lipid is preferably any substance according to formula 2, in which n=1, R₁ and R₂ is hydroxyacyl, R₃ is a hydrogen atom and R₄ is a 2-trimethylammonioethyl (the last compound corresponding to the phosphatidylcholine headgroup), 2-dimethylammonioethyl, 2-methylammonioethyl or 2-aminoethyl (corresponding to

a phosphatidylethanolamine headgroup).

A lipid of this kind is, for example, phosphatidylcholine from natural sources, in the old nomenclature also called lecithin. This can be obtained, for example, from eggs (then being rich in arachidic acid), soy-bean (rich in C-18 chains), coconuts (rich in saturated chains), olives (rich in monounsaturated chains), saffron, safflower and sunflowers (rich in n-6 linolenic acid), linseed (rich in n-3 linolenic acid), from whale-oil (rich in monounsaturated n-3 chains), from Nachtkerze or primrose (rich in n-3 chains), etc. Preferred natural phosphatidylethanolamines (in the old nomenclature also called cephalins), frequently stem from egg or soy-beans.

Further preferred lipids are synthetic phosphatidylcholines (R_4 in formula 2 corresponding to 2-trimethylammonioethyl), synthetic phosphatidylethanolamines (R_4 being identical to 2-aminoethyl), synthetic phosphatidic acids (R_4 being a proton) or their esters (R_4 corresponding e.g. to a short chain alkyl, such as methyl or ethyl), synthetic phosphatidylserines (R_4 corresponding to an L- or D-serine), or synthetic phosphatidyl(poly)alcohols, such as phosphatidylglycerol (R_4 being identical to L- or D-glycerol). In this case, R_1 and R_2 are identical acyloxy residues such as lauroyl, oleoyl, linoyl, linoleoyl or arachinoyl, e.g. dilauroyl-, dimyristoyl-, dipalmitoyl-, distearoyl-, diarachinoyl-, dioleoyl-, dilinoyl-, dilinoleoyl-, or diarachinoylphosphatidylcholine or -ethanolamine, or different acyl residues, e.g. R_1 = palmitoyl and R_4 = oleoyl, e.g. 1-palmitoyl-2-oleoyl-3-glycerophosphocholine; or different hydroxyacyl residues, e.g. R_1 = hydroxypalmitoyl and R_4 = hydroxyoleoyl; or mixtures thereof, e.g. R_1 = hydroxypalmitoyl and R_4 = oleoyl etc. R_1 can also signify an alkenyl and R_2 identical hydroxyalkyl residues, such as tetradecylhydroxy or hexadecylhydroxy, e.g.

in ditetradecyl- or dihexadecylphosphatidylcholine or -ethanolamine, R_1 can be an alkenyl and R_2 a hydroxyacyl, e.g. a plasmalogen (R_4 = trimethylammonioethyl), or R_1 can be an acyl, e.g. myristoyl, or palmitoyl, and R_2 a hydroxy, e.g. in natural or synthetic lysophosphatidylcholines or lysophosphatidylglycerols or lysophosphatidylethanolamines, e.g. 1-myristoyl- or 1-palmitoyllysophosphatidylcholine or -phosphatidylethanolamine; R_3 is frequently hydrogen.

A convenient lipid according to this invention is also a lipid of the basic formula 2, in which $n=1$, R_1 is an alkenyl residue, R_2 is an acylamido residue, R_3 is a hydrogen atom and R_4 is 2-trimethylammonioethyl (choline residue). A lipid of this kind is known under the term sphingomyeline.

Furthermore, suitable lipids are analogs of lysophosphatidylcholine, such as 1-lauroyl-1,3-propanediol-3-phosphorylcholine, monoglycerides, such as monoolein or monomyristin, a cerebroside, a ganglioside or a glyceride which contain no free or esterified phosphoryl- or phosphono group or a phosphino group in the position 3. One example of such glyceride is diacylglyceride or 1-alkenyl-1-hydroxy-2-acylglyceride with arbitrary acyl or alkenyl groups, the 3-hydroxy group in these then being ether-bonded to one of the mentioned carbohydrate residues, such as a galactosyl residue, for example in monogalactosylglycerol.

Lipids with desired head or chain group properties can also be prepared biochemically, using e.g. phospholipases (such as phospholipase A1, A2, B, C, and especially D), desaturases, elongases, acyl-transferases, etc., starting with any natural or synthetic precursor.

Suitable lipids, furthermore, are all lipids found in

biological membranes and extractable with suitable apolar organic solvents, such as chloroform. In addition to the lipids already mentioned, this group of lipids also encompasses steroids, such as oestradiols, or sterines, such as cholesterol, beta-sitosterine, desmosterine, 7-keto-cholesterol or beta-cholestanol, fat-soluble vitamins, such as retinoids, vitamins, such as vitamin A1 or A2, vitamin E, vitamin K, such as vitamin K1 or K2, or vitamin D1 or D3, etc.

EDGE ACTIVE SUBSTANCES

An edge active substance according to this application is any substance which is capable of inducing or increasing the carrier system's capacity to form edges, protrusions or relatively strongly curved surfaces; this property also manifests itself in the capability to induce pores in lipid structures, such as membranes, or even provoke a solubilization (lysis) in the higher concentrations ranges. More strictly speaking, all such substances are considered edge-active which exhibit a tendency to accumulate at or near the edges between the polar and apolar parts of molecules and/or near or at the edges between the polar and apolar parts of the supramolecular aggregates, thereby lowering the free energy for the formation of edges and/or strongly curved surfaces. All surfactants and many solvents as well as asymmetric, and thus amphiphatic, molecules or polymers, such as many oligo- and polycarbohydrates, oligo- and polypeptides, oligo- and polynucleotides or their derivatives also belong to this category.

The edge activity of the used 'solvents', surfactants, lipids, or agents depends on the effective relative hydrophilicity or hydrophobicity of each molecule, and can also be modified by the choice of further system components and boundary conditions in the system (temperature, salt content, pH value,

etc.). Functional groups, such as double bonds in the hydrophobic part of molecules, which lower the hydrophobicity of this molecular region, increase edge activity; elongation or space-demanding substituents in the hydrophobic molecular parts, e.g. in the aromatic part, lower the edge activity of a substance. Charged or strongly polar groups in the headgroup normally increase the edge activity provided that the hydrophobic molecular part has remained the same. Direct connections between the lipophilic and/or amphiphilic system components have the reverse effect.

Solvents which are to some extent edge active only in certain concentration ranges encompass simple, especially short chain, alcohols, such as methanol, ethanol, n-propanol, 2-propen-1-ol (allyl alcohol), n-butanol, 2-buten-1-ol, n-pentanol (amyl alcohol), n-hexanol, n-heptanol, n-octanol and n-decanol; furthermore, iso-propanol, iso-butanol or iso-pentanol. Higher alcohols are even more potent, for example, ethandiol (ethylene glycol), 1,2-propane diol (propylene glycol), 1,3-propane diol, 1,3-butane diol, 2,3-butane diol, propane triol (glycerol), 2-butene-1,4-diol, 1,2,4-butane triol, 1,3,4-butane triol, 1,2,3-butane triol, butane tetraol (erythritol), 2,2-bis(hydroxymethyl)1,3-propane diol (pentaerythritol), 2,4-pentadiol and other pentadiols or pentendiols, 1,2,5-pentantriol and other pentantriols or pententriols, pentantetraol, 1,2,6-hexane triol and other hexane triols, hexane tetraol and -pentaol, heptane diol, - triol, -tetraol, -pentaol and -hexaol, 1,4-butane diol- diglycidyl-ether, etc. Short-chain, di-, tri-, tetra-, penta- and hexa-oxyethylene glycols and -ethylene glycols are also suitable for the present purpose as well as cyclic alcohols, such as benzyl alcohol, cyclopentanol, cyclohexanol, 3-, 4-, 5-cyclohexanol, cyclohexyl alcohol, aryl-alcohols, such as phenyl-ethanol, etc.

Edge active solvents which can be used according to this invention include, furthermore, short-chain acyl-, alkyl-, alkenyl, hydroxyacyl-, alkenyloxy- as well as aryl derivatives of different acids and bases, such as acetic acid, formic acid, propionic acid, butenoic acid, pentenoic acid, etc. of many amino acids, benzoic acid, phosphoric- and sulphuric acid, of ammonia, purine, pyrimidine, etc., provided that they do not impair the chemical integrity of the carriers and the agent molecules to an unacceptable extent.

A nonionic edge active substance is any material which contains at least one, and in the majority of cases several, strongly hydrophilic groups and at least one, sometimes also several relatively hydrophobic, water insoluble residues. 'Nonionic' edge active substances can be zwitterionic or truly non-ionic.

Free of any charge and edge active are e.g. the lipoidal substances of the basic formula 3

$$R_1 - ((X_i - Y_j)_k - Z_l)_m - R_2 \quad (3)$$

in which X, Y and Z are different polar (hydrophilic) or apolar (hydrophobic) groups, which confer an amphiphatic character to the whole molecule. Z ist mainly a water soluble residue and i, j, k, l and m are greater or equal zero. R_1 and R_2 are two arbitrary residues; the first is mostly polar or very short; the second apolar.

The residues R_2 or X in such lipids often represent an acyl-, alkyl-, alkenyl-, hydroxyalkyl-, hydroxyalkenyl- or hydroxyacyl-chain with 8-24 carbon atoms. Very frequently, n-hexyl, n-heptyl, n-octyl, n-nonyl, n-decyl, n-undecyl, n-dodecyl, n-tetradecyl or n-tetradecenoyl, n-hexadecyl, n-

hexadecenoyl, n-octadecyl, n-octadecenoyl and n-octadecendienyl, n-octadecentrienyl, etc. are used.

Sorbitol is one possible example of residue Z. $(X_i - Y_j)$ can be a polyene, polyoxyalkene, such as polyoxyethylene, polyalcohol, such as polyglycol, or polyether. $(X_i - Y_j)$ mainly contain 1-20 and very frequently 2-10 units, e.g. in ethylene glycol, di- and triglycol (oligoglycol) or polyethylene glycol.

In simple substances according to formula 3, the residue R_1 or R_2 is frequently an alkyl-, alkenyl-, hydroxyalkyl-, alkenylhydroxy- or hydroxyacyl-chain with 1-24 carbon atoms. Very suitable are substances such as n-dodecyl (lauryl-ether), n-tetradecyl (myristoyl-ether), n-pentadecyl (cetyl-ether), n-hexadecyl (palmitoyl-ether), n-octadecyl (stearoyl-ether), n-tetradecenoyl (myristoleoyl-ether), n-hexadecenoyl (palmitoleoyl-ether) or n-octadecenoyl (oleoyl-ether). Owing to their good availability, the following substances are, amongst others, frequently used: 4-lauryl-ether (BrijTM 30), 9-lauryl-ether, 10-lauryl-ether, 23-lauryl-ether (Brij 35), 2-cetyl-ether (Brij 52), 10-cetyl-ether (Brij 56), 20-cetyl-ether (Brij 58), 2-stearyl-ether (Brij 72), 10-stearyl-ether (Brij 76), 20-stearyl-ether (Brij 78), 21-stearyl-ether (Brij 721), 2-oleoyl-ether (Brij 92), 10-oleoyl-ether (Brij 96) and 20-oleoyl-ether (Brij 78), the increasing number in their names indicating an increasing headgroup length. Suitable substances of this class are marketed under the names GENAPOLTM, THESITTM and LUBROLTM.

Amongst the most common nonionic surfactants of the ether-type which are suitable for the present purpose are the substances of the Myrj trademark, such as polyoxyethylene(8)-stearate (Myrj45), polyoxyethylene(20)-stearate (Myrj49), polyoxy-

ethylene(30)-stearate (MyrjTM51), polyoxyethylene(40)-stearate (Myrj52), polyoxyethylene(50)-stearate (Myrj53), polyoxyethylene(100)-stearate (Myrj59), etc. Further products of these classes are sold under the trademark Cirrasol ALN; common polyoxyethylene-alkylamides are e.g. surfactants of the trademark Atplus.

Another important special form of the nonionic edge active substance according to basic formula 3 most frequently contains a hydroxyl group in the position of residue R_1 and a hydrogen atom in the position of residue R_2 , by and large. Residues X and Z are frequently an alkoxy- or alkenoxy-, in principle also a hydroxyalkyl-, hydroxyalkenyl- or hydroxyacyl-chain with 4-100 carbon atoms. Residue Y, too, is frequently an alkoxy-, alkenoxy-, hydroxyalkyl-, hydroxyalkenyl- or hydroxyacyl-chain but one which is often branched and carries one methyl-or ethyl-side chain. Perhaps the most widely used edge active substances of this class are the surfactants which are marketed under the trademark "Pluronic".

Further, very commonly used special forms of non-ionic edge active substances are sold under the trademark "TWEEN". The cyclic part of this substance class is frequently a sorbitol ring. Residues R_1 , R_2 , R_3 and R_4 are frequently of the alkoxy- or alkenoxy-, and even more commonly of the polyene-, polyoxyalkene-, such as polyoxyethylene-, polyalcohol-, such as polyglycol-, or polyether type. Some of these chains can be apolar, corresponding to e.g. an acyl-, alkyl-, alkenyl-, hydroxyalkyl-, hydroxyalkenyl- or hydroxyacyl-chain with 8-24 carbon atoms. If none of residues R_1 , R_2 , R_3 or R_4 is apolar, one of the side-chains of a branched chain or one of the termini must be hydrophobic.

Chains in the substances of TWEEN type are very frequently of

the polyoxyethylene class. They mainly contain one terminal hydrogen atom and more rarely a methoxy group. One of the polyoxyethylene chains, however, contains a hydrophobic residue which preferably corresponds to an acyl-, alkyl-, alkenyl-, hydroxyalkyl-, hydroxyalkenyl- or hydroxyacyl-chain with 4-24, and in particular 12-18 carbon atoms.

Edge active substances which are sold under the trademark "TRITON" are also useful according to this invention.

Polyalcohol residues R_2 are most frequently esterified or etherified; however, in some cases they can also be bound to the hydrophobic chain through a nitrogen atom. They are very often adducts of ethyleneglycol, glycerol, erythritol, or pentaerythritol, for example 1-alkyl-, 1-alkenyl-, 1-hydroxyalkene-glycerol, or corresponding 1,2-, or 1,3-diglycerides (for example, 1-alkyl,2-alkyl-, 1-alkyl,2-alkenyl-, 1-alkenyl,2-alkyl-, 1-alkenyl,2-alkenyl-, 1-alkenyl,2-hydroxyalkyl-, 1-hydroxyalkyl,2-alkenyl-, 1-alkyl,2-hydroxyalkyl-, 1-hydroxyalkyl,2-alkyl-, 1-alkenyl,2-hydroxyalkene-, 1-hydroxyalkene,3-alkenyl-, 1-alkyl,3-alkyl-, 1-alkyl,3-alkenyl-, 1-alkenyl,3-alkyl-, 1-alkenyl,3-alkenyl-, 1-alkenyl,3-hydroxyalkyl-, 1-hydroxyalkyl,3-alkenyl-, 1-alkyl,3-hydroxyalkyl-, 1-hydroxyalkyl,3-alkyl-, 1-alkenyl,3-hydroxyalkene- or 1-hydroxyalkene,3-alkenyl-). Glycerol can be replaced by another oligo- or polyalcohol, such as erythritol, pentantriol, hexantriol, -tetraol or -pentaol, etc., resulting in a wide variety of linkage possibilities.

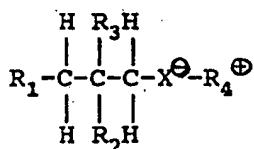
Z or R_2 , moreover, can contain one or more 1-10, preferably 1-6, most frequently 1-3 carbohydrate residues or their derivatives. 'Carbohydrate residue' in this context has the meaning as already described and is an alpha or beta and L- or D-alloside, -altroside, -fucoside, -furanoside, -galactoside,

-galactopyranoside, -glucoside, -glucopyranoside, -lactopyranoside, -mannoside, -mannopyranoside, -psicoside, sorboside, -tagatoside, -taloside; frequently used derivatives of disaccharides are L- or D-maltopyranoside, -maltoside, -lactoside, malto- or -lactobionamide; the corresponding derivatives of maltotriose or -tetraose are also useful.

The carbohydrate residue can also contain a sulfur atom, e.g. in beta-L- or D-thioglucopyranoside or -thioglycoside.

Zwitterionic surfactants are substances, for example, which contain a sulphonate group, such as (3-((3-cholamidopropyl)-dimethyliammonio)-1-propanesulfonate (CHAPS) and (3-((3-cholamidopropyl)-dimethyliammonio)-2-hydroxy-1-propane-sulfonate (CHAPSO) or N-octyl-N,N-dimethyl-3-ammonio-1-propane-sulfonate, N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate (lauryl-sulfobetaine), N-tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate (myristyl-sulfobetaine), N-hexadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate (palmityl-sulfobetaine), N-octadecyl-N,N-dimethyl-3-ammonio-1-propane-sulfonate (stearyl-sulfobetaine), N-octadecenoyl-N,N-dimethyl-3-ammonio-1-propanesulfonate (oleoyl-sulfobetaine) etc.

Zwitterionic surfactants are also substances with the basic formula 4



(4)

in which n is one or zero. One of both side chains R_1 and R_2 contains one acyl-, alkyl-, alkenyl-, alkenoyl-, hydroxyalkyl-, hydroxyalkenyl- or hydroxyacyl-, or alkoxy chain with 8-24 carbon atoms each; the other residue corresponds to a hydrogen, to a hydroxy group or to a short chain alkyl

residue. R_3 normally represents a hydrogen atom or a short alkyl chain. X is most frequently anionic, e.g. in a phosphate- or sulfate-residue. The residue R_4 in this case is cationic, in order to ensure that the whole molecule is zwitterionic. Most frequently, ammonio-alkyl derivatives, such as ethanol-, propanol-, butanol-, pentanolamine, hexanolamine, heptanolamine or octanolamine, N-methyl-, N,N-dimethyl, or N,N,N-trimethyl-ammonio-alkyl, N-ethyl-, N,N-diethyl, or N,N,N-triethyl-amino-alkyl, unequal N-alkyles, such as N,N-methyl-ethyl-ammonio-alkyl, or corresponding hydroxyalkyl substances are used, sometimes in a substituted form. (Single chain (lyso) derivatives of all biological zwitterionic phospholipids as well as their modified forms (such as Platelet-Activating-Factor and its analogs) also belong to this category.) R_4 can also be a positively charged carbohydrate residue, such as an aminosugar or one of its derivatives. R_4 and X, moreover, can exchange positions.

An ionic edge active substance is any material which contains at least one positive or negative charge and at least one segment which is poorly water soluble. An anionic substance of this kind can also contain several charges but must have a negative total charge. The total charge of any cationic substance must be positive.

Anionic edge active substances are for example the substances described by the basic formula 5:



in which R_1 is an organic hydrocarbon residue, which can also be substituted, and G^+ is a monovalent counterion, chiefly an alkali metal cation (such as lithium, sodium, potassium,

rubidium, or cesium), an ammonium ion or a low weight tetraalkylammonium-ion, such as tetramethylammonium or tetraethylammonium.

The hydrocarbon residue R_1 in an anionic surfactant of the basic formula 5 is frequently a straight chain or branched acyl, alkyl or alkenoyl, or oxidized or hydroxygenated derivative thereof; the residue R_1 can also contain one or several cyclic segments.

R_1 chain frequently contains 6-24, more frequently 10-20, and most frequently 12-18 carbon atoms; if unsaturated, it contains 1-6, and even more frequently 1-3, double bonds in n-3- or n-6- position.

The following hydroxyalkyl chains are preferred for the present purpose: n-dodecylhydroxy (hydroxylauryl), n-tetradecylhydroxy (hydroxymyristyl), n-hexadecylhydroxy (hydroxycetyl), n-octadecylhydroxy (hydroxystearyl), n-eicosylhydroxy or n-docosylhydroxy. Amongst the hydroxyacyl chains, the hydroxylauroyl, hydroxymyristoyl, hydroxypalmitoyl, hydroxystearyl, eicosylhydroxy or docosylhydroxy chains are especially worth mentioning; particularly interesting amongst the hydroxyalkene-residues are the hydroxydodecen, hydroxytetradecen, hydroxyhexadecen, hydroxyoctadecen, hydroxyeicosen, hydroxydocosen, most notably 9-cis,12-hydroxy-octadecenyl (ricinolenyl) or 9-trans,12-hydroxy-octadecenyl (ricinelaidyl), 5-cis,8-cis,11-cis,14-cis,15-hydroxy-eicosatetraenyl (15-hydroxy-arachidonyl), 5-cis,8-cis,11-cis,14-cis,15-hydroxy,17-cis-eicosapentaenyl, 4-cis,7-cis,10-cis,13-cis,15-hydroxy,16-cis-docosapentaenyl and 4-cis,7-cis,10-cis,13-cis,15-hydroxy,16-cis,19-cis-docosahexaenyl.

Another class of anionic, edge active substances corresponds

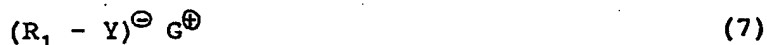
to basic formula 6



here, R_1 is a hydrocarbon residue which can also be substituted; X is a short-chain alkyl residue and Y denotes a sulfonate-, sulfate-, phosphate-, phosphonate or phosphinate group. G^+ is a mostly monovalent counterion (cation).

Alkali metal alkyl- or -alkenylethersulfonates or -phosphates belong to this class of ether-bonded molecules. Special examples are sodium- or potassium-n-dodecyloxyethylsulfate, -n-tetradecyloxyethylsulfate, -n-hexadecyl-oxyethylsulfate or -n-octadecyloxyethylsulfate or an alkali metal alkane sulfonate, such as sodium- or potassium-n-hexanesulfonate, n-octansulfonate, n-decansulfonate, n-dodecansulfonate, -n-tetradecansulfonate, -n-hexadecansulfonate or -n-octadecansulfonate.

The substances of general formula 7



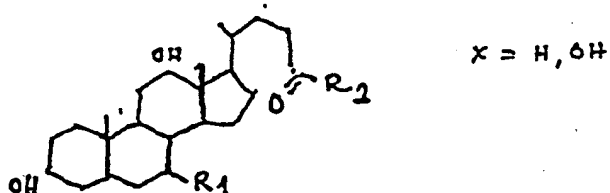
are related to the compounds of basic type 6. These are analogous to the substances of formula 6 but contain a directly (covalently) coupled charged headgroup.

Particularly useful anionic, edge active substances of above formula 6 are alkali metal-alkylsulfates. To mention just a few examples: sodium or potassium-n-dodecyl (lauryl)-sulfate, -n-tetradecyl (myristyl)-sulfate, -n-hexadecyl (palmityl)-sulfate, -n-octadecyl (stearyl)-sulfate, n-hexadecylen (palmitolein)-sulfate and n-octadecylen (olein)-sulfate. Instead of a sulfate group, sulfonate, n-methyl- or n-ethylglycine for example can also be used.

Various salts of bis-(2-alkyl-alkyl)-sulfosuccinate are also suitable for the applications as described in this work. Preferably, these are used as lithium-, sodium-, potassium-, or tetramethylammonium-bis-(2-ethyl-hexyl)-sulfosuccinate.

Furthermore, sarcosides, as well as alkyl- or alkenoyl-sulfochloride derivatives of the protein condensates, sulfonamide soaps, sulfatated or phosphorylated alcohol-esters, sulfatated or phosphorylated amides or monoglycerides, moreover, fatty acid alkylamides, sulfo- or phospho-succinic acid esters, taurides, alkylphenol-, alkylbenzol-, alkyl-naphthalene-ethersulfonates etc., are also all useful.

Another important group of anionic edge active substances are the derivatives of cholic acid. Their basic formula reads



here, R_1 corresponds to a proton, an OH- or a carbonyl group and R_2 can be a derivative of taurine or glycocoll, for example. Particularly suitable are various salts of cholic acid (bile acid, 3 α ,7 α ,12 α -trihydroxy-5 β -cholane-24-oic-acid), deoxycholic acid (3 α ,12 α -dihydroxy-5 β -cholane-24-oic-acid), chenodeoxycholic acid, glycocholic acid (N-(3 α ,7 α ,12 α -trihydroxy-24-oxycholane-24-yl)-glycine), deoxycholic acid, glycodeoxycholic acid (N-(3 α ,12 α -dihydroxy-24-oxycholane-24-yl)-glycine), glychenodeoxycholic acid, glycolithocholic acid, glycoursodeoxycholic acid, lithocholic acid, taurodeoxycholic

acid, taurocholic acid (3alpha,7alpha,12alpha-trihydroxy-5beta-cholan-24-oin-acid-N-(sulfoethyl)amide), taurochenodeoxycholic acid, tauroglycocholic acid, taurolitocholic acid, taurolitocholic acid-3-sulfate, tauroursodeoxycholic acid, ursocholanic acid, ursodeoxycholic acid (3alpha,7beta-dihydroxy-5beta-cholanic acid), the most common counterions being sodium or potassium.

Diverse cholic acid esters, such as cholesteryl-alkyl-, -alkenyl-, -hydroxyalkyl-, -hydroxyalkene-esters or cholesterylsulfates and -sulfonates are also edge active according to this invention.

Related synthetic adducts of the CHAPS class can also be used; in this case, R_2 is frequently an $NH-(CH_2)_3-N',N'-(CH_2)_2(CH_2)_2-R_3-CH_2-SO_3$ segment, whilst R_3 can be a proton or a carbonyl group. Again, sodium or potassium are the most commonly used counterions.

Digitonines as well as saponines, such as Quillaja acid, have similar basic structures in their cores as the cholic acid derivatives; consequently, they can also be used as edge active substances according to this invention.

The basic formula of the phosphorus-containing anionic edge active substances is



in which n is zero or one. One of the two side chains R_1 and R_2 contains hydrogen, a hydroxy group or a short chain alkyl residue; the other contains an alkyl-, alkenyl-, hydroxy-

alkyl-, hydroxyalkenyl- or hydroxyacyl-chain (or an alkenyl-, alkoxy-, alkenyloxy- or acyloxy-residue) with 8-24 carbon atoms. The R_3 residue, as a rule, corresponds to hydrogen or an alkyl chain with less than 5 carbon atoms. R_4 can be an anionic oxygen or a hydroxy group; an alkyl chain with up to 8 C-atoms can also appear as well as another carbohydrate residue with up to 12 carbon atoms; if R_1 as well as R_2 are hydrogen and/or hydroxy groups, a steroid residue, a sugar derivative, a chain containing an amino group, etc., can also appear. Alkyl residues can also be substituted.

Amongst the most suitable surfactants of this substance class are: n-tetradecyl(=myristoyl)-glycero-phosphatidic acid, n-hexadecyl(=plamityl)-glycero-phosphatidic acid, n-octadecyl(=stearyl)-glycero-phosphatidic acid, n-hexadecylene(=palmitoleil)-glycero-phosphatidic acid, n-octadecylene(=oleil)-glycero-phosphatidic acid, n-tetradecyl-glycero-phosphoglycerol, n-hexadecyl-glycero-phosphoglycerol, n-octadecylene-glycero-phosphoglycerol, n-tetradecyl-glycero-phosphoserine, n-hexadecyl-glycerophosphoserine, -n-octadecyl-glycero-phosphoserine, n-hexadecylene-glycero-phosphoserine and n-octadecylene-glycero-phosphoserine.

The corresponding lyso-sulfolipids, phosphono- or phosphino-lipids are also suitable edge active compounds according to this invention.

Counterion in these compounds is most frequently an alkali metal cation (such as lithium, sodium, potassium, cesium) or a water soluble tetraalkylammonium-ion (such as tetramethylammonium, tetrathylammonium, etc.).

All corresponding statements made above for surfactants of basic formula 3 also pertain to the carbohydrate residue R_1 .

This residue in the majority of cases is a straight chain or branched alkyl or alkenoyl chain with 6-24, very frequently 10-20, in particular 12-18, carbon atoms and 1-6, especially frequently 1-3, double bonds in n-3- or n-6- positions.

Very convenient alkyl-residues R_1 or R_2 are, for example, n-dodecyl, n-tetradecyl, n-hexadecyl, n-octadecyl, n-eicosyl or n-docosyl chains. N-nonyl, n-undecyl, n-tridecyl, n-pentadecyl, n-heptadecyl and n-nonadecyl, however, are equally useful.

An alkenyl in position R_1 or R_2 is preferably a 9-cis-dodecenyl (lauroleyl), 9-cis-tetradecenyl (myristoleyl), 9-cis-hexadecenyl (palmitoleoyl), 6-cis-octadecenyl (petroselinyl), 6-trans-octadecenyl (petroselaidinyl), 9-cis-octadecenyl (oleyl), 9-trans-octadecenyl (elaidinyl), 11-cis-octadecenyl (vaccenyl), 9-cis-eicosenyl (gadoleinyl), 13-cis-docosenyl, 13-trans-docosenyl or 15-cis-tetracosenyl, etc.

Higher unsaturated alkenyls which also can be used for the present purpose are, amongst others: 9-cis,12-cis-octadecendienyl, 9-trans,12-trans-octadecendienyl, 9-cis,12-cis,15-cis-octadecentrienyl, 6-cis,9-cis,12-cis-octadecentrienyl, 11-cis,14-cis,17-cis-eicosatrienyl, 6-cis,9-cis,12-cis,15-cis-octadecentetraenyl, 5-cis,8-cis,11-cis,14-cis-eicosatetraenyl, 5-cis,8-cis,11-cis,14-cis,17-cis-eicosapentaenyl, 4-cis,7-cis,10-cis,13-cis,16-cis-docosapentaenyl and 4-cis,7-cis,10-cis,13-cis,16-cis,19-cis-docosahexaenyl.

R_1 and R_2 are preferably chosen from the substances of the hydroxyalkyl-class, in which case they correspond, for example, to n-decylhydroxy, n-dodecylhydroxy (hydroxylauryl), n-tetradecylhydroxy (hydroxymyristyl), n-hexadecylhydroxy (hydroxycetyl), n-octadecylhydroxy (hydroxystearyl) and n-

eicosylhydroxy (hydroxyarachinyl) chains.

An alkenylhydroxy in R_1 or R_2 is preferably a 9-cis-dodecenylhydroxy (hydroxylauroleyl), 9-cis-tetradecenylhydroxy (hydroxymyristoleyl), 9-cis-hexadecenylhydroxy (hydroxypalmitoleinyl), 6-cis-octadecenylhydroxy (petroselinylhydroxy), 6-trans-octadecenylhydroxy (hydroxypetroselaidinyl), 9-cis-octadecenylhydroxy (hydroxyoleyl), 9-trans-octadecenylhydroxy (hydroxyelaidinyl) and 9-cis-eicosenyl (hydroxygadoleinyl) chain.

An alkanoylhydroxy in R_1 or R_2 is preferably an n-decanoylhydroxy, n-dodecanoylhydroxy (lauroylhydroxy), n-tetradecanoylhydroxy (myristoylhydroxy), n-hexadecanoylhydroxy, n-hexadecanoylhydroxy (palmitoylhydroxy), n-octadecanoylhydroxy (stearoylhydroxy) and n-eicosoylhydroxy (arachinoylhydroxy) chain.

An alkenoylhydroxy in R_1 or R_2 is preferably a 9-cis-dodecenylhydroxy (lauroleoylhydroxy), 9-cis-tetradecenylhydroxy (myristoleoylhydroxy), 9-cis-hexadecenylhydroxy (palmitoleinoylhydroxy), 6-cis-octadecenylhydroxy (petroselinoylhydroxy), 6-trans-octadecenylhydroxy (petroselaidinoylhydroxy), 9-cis-octadecenylhydroxy (oleoylhydroxy), 9-trans-octadecenylhydroxy (elaidinoylhydroxy) and 9-cis-eicosenyl (gadoleinoylhydroxy) chain.

Some examples for the short chain alkyl residue, which often appear in the R_4 residue, are methylene-, ethylene-, n-propylene-, iso-propylene-, n-butylene- or iso-butylene- as well as n-pentylene- or n-hexylene-groups. R_4 can also be a carboxy- or a sulfo-group, an acid or alkaline group, such as carboxy- and amino-group; the amino group in such case is always in the alpha-position relative to the carboxy group.

Another example for the R_4 residue are free or etherified hydroxy groups (two ether-bonded hydroxy groups, in such case, can be connected by one divalent hydrocarbon residue, such as methylene, ethylene, ethylidene, 1,2-propylene or 2,2-propylene). R_4 , furthermore, can be substituted by a halogen atom, such as chlorine or bromine, a low weight alkoxy-carbonyl, such as methoxy- or ethoxycarbonyl, or by a low weight alkansulfonyl-, such as methansulfonyl.

A substituted short chain alkyl residue R_4 with 1-7 C-atoms is preferably carboxy-short-chain alkyl, such as carboxy-methyl, carboxyethyl- or 3-carboxy-n-propyl, omega-amino-n-carboxy- a short-chain alkyl, such as 2-amino-2-carboxyethyl or 3-amino-3-carboxy-n-propyl, hydroxy-short-chain alkyl, such as 2-hydroxyethyl or 2,3-dihydroxypropyl, a short-chain alkoxy-3-methoxy-n-propyl, a short-chain alkylendioxy-short-chain alkyl, such as 2,3-ethylenedioxypropyl or 2,3-(2,2-propylene)-dioxypropyl, or halogen-short-chain alkyl, such as chloro- or bromo-methyl, 2-chloro- or 2-bromo-ethyl, 2- or 3-chloro- or 2-or 3-bromo-n-propyl.

A carbohydrate residue R_4 with 5-12 C-atoms is, for example, a natural monosaccharide residue stemming from a pentose or a hexose in the aldose or ketose form.

A carbohydrate residue R_4 , moreover, can be a natural disaccharide residue, such as a disaccharide residue formed from two hexoses, in the described sense. A carbohydrate residue R_4 can also be a derivatised mono-, di- or oligosaccharide residue, in which an aldehyde group and/or one or two terminal hydroxy groups are oxidized to a carboxy group, e.g. a D-glucon-, D-glucar- or D-glucoron acid residue; this preferably appears in the form of a cyclic lactone residue. The aldehyde- or keto-groups in a derivatised mono-

or disaccharide residue can also be reduced to a hydroxy group, e.g. in inositol, sorbitol or D-mannitol; also, one or several hydroxy groups can be replaced by a hydrogen atom, e.g. in desoxysugars, such as 2-desoxy-D-ribose, L-rhamnose or L-fucose, or by an amino group, e.g. in aminosugars, such as D-glucosamine or D-galactosamine.

R_4 can also be a steroid residue or a sterine residue. If R_4 is a steroid residue, R_3 is a hydrogen atom, whilst R_1 and R_2 in such case preferably correspond to a hydroxy group.

The counterion in such cases is preferably an ammonium, sodium or potassium ion.

In an anionic surfactant of formula 8, the following values of parameters are preferred: $n = 1$, R_1 is an alkyl, such as n-dodecyl (lauryl), n-tridecyl, n-tetradecyl (myristyl), n-pentadecyl, n-hexadecyl (cetyl), n-heptadecyl or n-octadecyl (stearyl), hydroxyalkyl, such as n-dodecylhydroxy (hydroxylauryl), n-tetradecylhydroxy (hydroxymyristyl), n-hexadecylhydroxy (hydroxycetyl), or n-octadecylhydroxy (hydroxystearyl), hydroxyacyl, such as hydroxylauroyl, hydroxymyristoyl, hydroxypalmitoyl or hydroxystearoyl, R_2 is a hydrogen atom or a hydroxy group, R_3 is a hydrogen atom or a short-chain alkyl, such as methyl, R_4 is a short-chain alkyl, e.g. methyl or ethyl, short-chain alkyl substituted by an acid or an alkaline group, such as a carboxy and amino group, e.g. omega-amino-omega-carboxy-short-chain alkyl, such as 2-amino-2-carboxyethyl or 3-amino-3-carboxy-n-propyl, hydroxy-short-chain alkyl, such as 2-hydroxyethyl or 2,3-hydroxypropyl, short-chain alkylenedioxy-short-chain alkyl, e.g. 2,3-ethylenedioxypropyl or 2,3-(2,2-propylene)-dioxypropyl, halogen-short-chain alkyl, such as 2-chloro- or 2-bromo-ethyl group, a carbohydrate residue with 5-12 C-

atoms, e.g. in inositol, or a steroid residue, such as a sterol, e.g. cholesterol, and G^+ is a sodium-, potassium- or ammonium-ion.

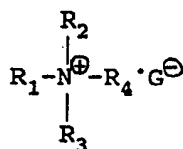
An anionic surfactant of formula 8, in many cases, is a sodium- or potassium salt of lysophosphatidylserine, such as the sodium- or potassium salt of lysophosphatidylserine from bovine brain or the sodium- or potassium salt of a synthetic lysophosphatidylserine, such as sodium- or potassium-1-myristoyl- or -1-palmitoyl-lysophosphatidylserine, or a sodium- or potassium salt of lysophosphatidylglycerols. The hydrogen atom on the phosphate group can be replaced by a second cation, G^+ or calcium-, magnesium-, manganese-ion, etc.

An anionic surfactant of formula 8 preferably contains an alkyl chain, such as n-dodecyl (lauryl), n-tridecyl, n-tetradecyl (myristoyl), n-pentadecyl, n-hexadecyl (cetyl), n-heptadecyl or n-octadecyl (stearyl), a hydroxyalkyl chain, such as n-dodecylhydroxy (hydroxylauryl), n-tetradecylhydroxy (hydroxymyristyl), n-hexadecylhydroxy (hydroxycetyl), or n-octadecylhydroxy (hydroxystearyl), a hydroxyacyl chain, such as hydroxylauroyl, hydroxymyristoyl, hydroxypalmitoyl or hydroxystearyl in position R_1 , a hydrogen atom or a hydroxy group in position R_2 , and a hydrogen atom or a short-chain alkyl, such as methyl group, in position R_3 . G^+ is preferably an ammonium, sodium, potassium or tetramethylammonium ion.

An anionic surfactant of formula 8 can, furthermore, be a sodium- or potassium salt of a natural phosphatidic acid, such as egg-phosphatidic acid, a sodium- or potassium salt of a natural lysophosphatidic acid, such as egg-lysophosphatidic acid, a sodium- or potassium salt of a synthetic lysophosphatidic acid, such as 1-lauroyl-, 1-myristoyl-, 1-palmitoyl- or 1-oleoyl-lysophosphatidic acid, etc.

The most important classes of cationic surfactants encompass: ammonium salts, quaternary ammonium salts, salts of heterocyclic bases, such as alkylpyridium-, imidazole-, or imidazolinium salts, salts of alkylamides and polyamines, salts of acylated diamines and polyamines, salts of acylated alkanolamines, salts of alkanolamine esters and ethers, etc.

A cationic surfactant is, for example, any substance corresponding to the formula:



(9)

in which R_1 is a hydrocarbon residue which can also be substituted. R_2 denotes a short-chain alkyl, phenyl-short-chain-alkyl or hydrogen atom. R_3 and R_4 correspond to a short-chain alkyl residue. R_2 and R_3 , together with the nitrogen atom, represent an aliphatic heterocycle, which can also be substituted on a carbon atom; R_4 is a short-chain alkyl; R_2 , R_3 and R_4 , together with the nitrogen atom, can also form an aromatic heterocycle, which, moreover, can be substituted on one of the carbon atoms. G^- corresponds to an anion.

In a cationic surfactant of basic formula 9, R_1 represents an aliphatic hydrocarbon residue, which can also be substituted, for example, by an aryloxy- short-chain-alkoxy-, a substituted short-chain alkyl, a straight chain or branched chain alkyl with 7-22, and in particular 12-20, carbon atoms, or an alkenyl with 8-20, or in particular 12-20, carbon atoms and 1-4 double bonds.

Particularly preferred for use are straight chain alkyls with an even number of 12-22 carbon atoms, such as n-dodecyl, n-tetradecyl, n-hexadecyl, n-octadecyl, n-eicosyl or n-docosyl.

An alkenyl with 8-24, in particular 12-22, carbon atoms and 0-5, in particular 1-3, double bonds is e.g. 1-octenyl, 1-nonenyl, 1-decenyl, 1-undecenyl, 1-dodecenyl, 9-cis-dodecenyl (lauroleyl), 1-tridecenyl, 1-tetradecenyl, 9-cis-tetradecenyl (myristoleyl), 1-pentadecenyl, 1-hexadecenyl, 9-cis-hexadecenyl (palmitoleinyl), 1-heptadecenyl, 1-octadecenyl, 6-cis-octadecenyl (petroselinyl), 6-trans-octadecenyl (petroselaidinyl), 9-cis-octadecenyl (oleyl), 9-trans-octadecenyl (elaidinyl), 9-cis-12-cis-octadecadienyl (linoleyl), 9-cis-11-trans-13-trans-octadecatrienyl (alpha-elaostearinyl), 9-trans-11-trans-13-trans-octadecatrienyl (beta-elaostearinyl), 9-cis-12-15-cis-octadecatrienyl (linolenyl), 9-, 11-, 13-, 15-octadecatetraenyl (parinaryl), 1-nonadecenyl, 1-eicosenyl, 9-cis-eicosenyl (gadoleinyl), 5-, 11-, 14-eicosatrienyl or 5-, 8-, 11-, 14-eicosatetraenyl (arachidonyl).

Preferred alkenyls contain 12-20 carbon atoms and one double bond, e.g. 9-cis-dodecenyl (lauroleyl), 9-cis-tetradecenyl (myristoleyl), 9-cis-hexadecenyl (palmitoleinyl), 6-cis-octadecenyl (petroselinyl), 6-trans-octadecenyl (petroselaidinyl), 9-cis-octadecenyl (oleyl), 9-trans-octadecenyl (elaidinyl) or 9-cis-eicosenyl (gadoleinyl).

Methyl or ethyl are two examples of short-chain alkyl residues R_2 , R_3 or R_4 which appear in substances of formula 9.

Two examples of phenyl-short-chain-alkyl groups in R_2 are benzyl or 2-phenylethyl.

An aliphatic heterocycle, which can form from R_2 and R_3 together with the nitrogen atom is, for example, a monocyclic, five- or six-member aza-, oxaaza- or thiazacyclic residue, as in piperidino, morpholino or thiamorpholinio groups.

Substituents of this heterocycle are the substituents R_1 and R_4 on the nitrogen as well as, in some cases, on the carbon atom; they are, most frequently, of the short-chain alkyl, such as methyl, ethyl, n-propyl or n-butyl type.

A heterocycle, which is formed from R_2 and R_3 together with nitrogen and is substituted on a carbon atom through a short-chain alkyl, is e.g. of the 2-, 3- or 4-methylpiperidinio, 2-, 3- or 4-ethylpiperidinio or 2- or 3-methylmorpholinio type.

An aromatic heterocycle, formed from R_2 , R_3 and R_4 together with the nitrogen atom, is, for example, a monocyclic five- or six-member aza-, diaza-, oxaaza- or thiazacyclic residue, such as pyridinio, imidazolinio, oxazolinio or thiazolinio or, for example, a benzocondensed monoazabicyclic residue, such as chinolinio or iso-chinolinio group.

Substituents of such heterocycles are the residue R_1 on the nitrogen atom as well as a short-chain alkyl, such as methyl or ethyl, hydroxy-short-chain alkyl, such as hydroxymethyl or 2-hydroxyethyl, oxo-, hydroxy- or halogen, such as chloro- or bromo-compounds, which can also be substituted on a carbon atom.

A heterocycle, formed from R_2 , R_3 and R_4 and substituted on a carbon atom through the mentioned residues is, for example, a 2- or 4-short-chain-alkylpyridinio, e.g. 2- or 4-methyl or 2- or 4-ethylpyridinio, di-short-chain-alkylpyridinio, e.g. 2,6-

dimethyl-, 2-methyl-3-ethyl-, 2-methyl-4-ethyl-, 2-methyl-5-ethyl-, or 2-methyl-6-ethylpyridinio, 2-, 3- or 4-halogen-pyridinio, e.g. 2-, 3- or 4-chloropyridinio or 2-, 3- or 4-bromo-pyridinio, 2-short-chain alkylimidazolinio, -oxazolinio or -thiazolinio, such as 2-methyl- or 2-ethylimidazolinio, -oxazolinio or -thiazolinio or 2-short-chain alkyl-8-halogenchinolinio, such as 2-methyl-8-chlorochinolinio group.

A cationic surfactant of formula 9 is preferably an N-benzyl-N,N-dimethyl-N-2-(2-(4-(1,1,3,3-tetramethylbutyl)-phenhydroxy)-ethhydroxy)-ethylammoniochloride, N-benzyl-N,N-dimethyl-N-2-(2-(3(methyl-4-(1,1,3,3-tetramethylbutyl)-phenhydroxy)-ethhydroxy)-ethylammoniochloride (methylbenzethoniumchloride), n-dodecyltrimethylammoniochloride or -bromide, trimethyl-n-tetradecylammoniochloride or -bromide, n-hexadecyltrimethylammoniochloride or -bromide (cetyltrimethyl-ammoniumchloride or -bromide), trimethyl-n-octadecylammoniochloride or -bromide, ethyl-n-dodecyl-dimethylammoniochloride or -bromide, ethyldimethyl-n-tetradecylammoniochloride or -bromide, ethyl-n-hexadecyldimethylammoniochloride or -bromide, ethyldimethyl-n-octadecylammoniochloride or -bromide, n-alkyl-benzyl-dimethyl-ammoniochloride or -bromid (benzalkoniumchloride or -bromide), such as benzyl-n-dodecyldimethylammoniochloride or bromide, benzyldimethyl-n-tetradecylammoniochloride or -bromide, benzyl-n-hexadecyldimethyl-ammoniochloride or -bromide or benzyldimethyl-n-octadecylammonio-chloride or -bromide, N-(n-decyl)-pyridiniochloride or -bromide, N-(n-dodecyl)-pyridiniochloride or -bromide, N-(n-tetradeyl)-pyridiniochloride or -bromide, N-(n-hexadecyl)-pyridiniochloride or -bromide (cetylpyridiniumchloride) or N-(n-octadecyl)-pyridinio-chloride or -bromide. Mixtures of these or other edge active substances are also suitable.

The following surfactants are especially useful for biological

purposes: N,N-bis(3-D-glucon-amidopropyl)cholamide (BigCHAP), Bis(2-ethylhexyl)sodium-sulfosuccinate, cetyl-trimethyl-ammonium-bromide, 3-((cholamidopropyl)-dimethylammonio)-2-hydroxy-1-propane sulfonate (CHAPSO), 3-((cholamidopropyl)-dimethylammonio)-1-propane sulfonate (CHAPS), cholate-sodium salt, decaoxyethylene-dodecyl-ether (Genapol C-100), decaethylene-isotridecyl-ether (Genapol X-100), decanoyl-N-methyl-glucamide (MEGATM-10), decyl-glucoside, decyl-maltoside, 3-(decyldimethylammonio)-propane-sulfonate (Zwittergent 3-10), deoxy-bigCHAP, deoxycholate, sodium salt, digitonin, 3-(dodecyldimethylammonio)-propane-sulfonate (Zwittergent 3-12), dodecyl-dimethyl-amine-oxide (EMPIGENTM), dodecyl-maltoside, dodecylsulfate, glyco-cholate, sodium salt, glyco-deoxycholate, sodium salt, heptaethylene-glycol-octyl-phenyl-ether (tritonTM X-114), heptyl-glucoside, heptyl-thioglucoside, 3-(hexadecyldimethylammonio)-propane-sulfonate (Zwittergent 3-14), hexyl-glucoside, dodecyl-dimethyl-amine-oxide (GenaminoxTM KC), N-dodecyl-N,N-dimethylglycine (Empigen BB), N-decyl-sulfobetaine (Zwittergent 3-10), N-dodecyl-sulfobetaine (Zwittergent 3-12), N-hexadecyl-sulfobetaine (Zwittergent 3-16), N-tetradecyl-sulfobetaine (Zwittergent 3-14), N-octyl-sulfobetaine (Zwittergent 3-08), nonaethylene-glycol-mono-dodecyl-ether (THESIT), nonaethylene-glycol-octyl-phenol-ether (triton X-100), nonaethylene-glycol-octyl-phenyl-ether (NP-40, NonidetTM P-40), nonaethylene-dodecyl-ether, nonanoyl-N-methyl-glucamide (MEGA-9), nonaoxyethylene-dodecyl-ether (Lubrol PX, Thesit), nonyl-glucoside, octaethylene-glycol-isotridecyl-ether (Genapol X-080), octaethylene-dodecyl-ether, octanoyl-N-methyl-glucamide (MEGA-8), 3-(octyldimethylammonio)-propane-sulfonate (Zwittergent 3-08), octyl-glucoside, octyl-thioglucoside, entadecaethylene-isotridecyl-ether (Genapol X-150), polyethylene-polypropylene-glycol (Pluronic F-127), polyoxyethylene-sorbitane-monolaurate (Tween 20), polyoxyethylene-sorbitane-monooleate (Tween 80), taurodeoxycholate-sodium salt, taurocholate-sodium salt, 3-(tetradecyl-

dimethylammonio)-propane-sulfonate (Zwittergent 3-14), etc.

Particularly suitable for pharmacological purposes are:
cetyl-trimethyl-ammonium-salts (such as hexadecyltrimethylammoniumbromide, trimethylhexadecylamine-bromo-salt), cetylsulfate salts (such as Na-salt, Lanette™ E), cholate salts (such as Na- and ammonium-form) decaoxyethylene-dodecyl-ether (Genapol™ C-100), deoxycholate salts, dodecyl-dimethyl-amine-oxide (Genaminox KC, EMPIGEN), N-dodecyl-N,N-dimethylglycine (Empigen BB), 3-(hexadecyldimethylammonio)-propane-sulfonate (Zwittergent™ 3-14), fatty acid salts and fatty alcohols, glyco-deoxycholate salts, laurylsulfate salts (sodium dodecylsulfate, Duponol™ C, SDS, Texapon™ K12), N-hexadecyl-sulfobetaine (Zwittergent 3-16), nonaethylene-glycol-octyl-phenyl-ether (NP-40, Nonidet P-40), nonaethylene-dodecyl-ether, octaethylene-glycol-isotridecyl-ether (Genapol X-080), octaethylene-dodecyl-ether, polyethylene glycol-20-sorbitane-monolaurate (Tween 20), polyethylene glycol-20-sorbitane-monostearate (Tween 60), polyethylene glycol-20-sorbitane-monooleate (Tween 80), polyhydroxyethylene-cetylstearylether (Cetomacrogol™, Cremophor™ O, Eumulgin™, C 1000) polyhydroxyethylene-4-laurylether (Brij 30), polyhydroxyethylene-23-laurylether (Brij 35), polyhydroxyethylene-8-stearate (Myrj 45, Cremophor AP), polyhydroxyethylene-40-stearate (Myrj 52), polyhydroxyethylene-100-stearate (Myrj 59), polyethoxylated castor oil 40 (Cremophor EL), polyethoxylated hydrogenated castor oil (Cremophor RH 40, Cremophor RH 60) polyethoxylated plant oils (Lebrafils™), sorbitane-monolaurate (Arlacel 20, Span 20), taurodeoxycholate salts, taurocholate salts, polyethylene glycol-20-sorbitane-palmitate (Tween 40), Myrj 49 and polyethylene glycol derivatives of ricinols, etc.

AGENTS:

Transfersomes as described in this invention are suitable for the application of many different agents and, in particular, for therapeutic purposes, for example. The preparations according to this invention can contain the following:

- at least one adrenocorticostatic agent, in particular metyrapon;
- at least one carrier substance, additive or agent, belonging to the class of beta-adrenolytics (beta blocking agents), very frequently acetobol, alprenolol, bisoprololfumarate, bupranolol, carazolol, celiprolol, mepindolsulfate, metipranolol, metoprolotartat, nadolol, oxyprenolol, pindolol, sotalol, tertatolol, timolohydrogen maleate and toliprolol, especially preferred, atenolol or propranolol;
- at least one carrier substance, additive or agent, belonging to the androgenes or antiandrogenes, in particular drostanolonpropionate, mesterolone, testosteroneundecanoate, testolacton, yohimbine, or chloroamidinonacetate, cyproteronacetate, ethinylestradiol or flutamide;
- at least one carrier substance, additive or agent with an antiparasitic action, frequently phanquinone, benzyobenzoate, bephenium-hydroxy-naphthoate, crotamiton, diethylcarbamazine, levamisol, lindane, malathion, mesulfene (2,7-dimethylantren), metronidazol or tetramisol;
- at least one anabolic agent, in particular clostebolacetate, cyanocobolamine, folic acid, mestanolone, metandienone, metenolone, nandrolone, nandrolondecanoate, nandrolone-hexyloxyphenylpropionate,

nandrolon-phenyl-propionate, norethandrolone, oxaboloncipionate, piridoxine or stanozolole;

- at least one agent which can induce systemic anesthesia or analgesia, e.g. chlorobutanol, ketamine, oxetacaine, propanidide and thiamylal, aminophenol-derivatives, aminophenazol-derivatives, antranilic acid- and arylpropione acid derivatives, azapropazone, bumadizone, chloroquin- and codeine-derivatives, diclophenac, fentanil, ibuprofen, indometacine, ketoprofen, methadone-substances, morazone, morphine and its derivatives, nifenazone, niflumin acid, pentazozine, pethidine, phenazopyridine, phenylbutazone-derivatives (such as 3,5 pyrazolidine dion), pherazone, piroxicam, propoxyphene, propyphenazon, pyrazol- and phenazone-derivatives (aminophenazone, metamizole, monophenylbutazone, oxyphenebutazone, phenylbutazone or phenazone-salyzilate), salicylic acid-derivatives, sulfasalazine, tilidine; acetylsalicylic acid, ethylmorphine, alclofenac, alphaprodine, aminophenazone, anileridine, azapropazone, benfotiamine, benorilate, benzydamine, cetobemidone, chlorophenesincarbamate, chlorothenoxazine, codeine, dextromoramide, dextro-propoxyphene, ethoheptazine, fentanyl, fenylramidol, fursultiamine, flupirtinmaleate, glafenine, hydromorphone, lactylphenetidine, levorphanol, mefenamic acid, meptazonol, methadone, mofebutazone, nalbufine, Na-salt of noramidopyrinium-methanesulfonate, nefopam, normethadone, oxycodone, paracetamol, pentazocine, pethidine, phenacetine, phenazocine, phenoperidine, pholcodine, piperylone, piritramide, procaine, propyphenazone, salicylamide, thebacone, tiemonium-odide, tramadone;

- at least one substance from the class of analeptics, such

as aminophenazole, bemegride, caffeine, doxapram, ephedrine, prolintane, or nialamide and tranylcypromine; but also vitamins, plant extracts from semen colae, camphor, menthol;

- at least one substance from the class of antiallergics: e.g. agents from the globuline family, corticoids or antihistaminics (such as beclometasone-, betametasone-cortisone-, dexametasone-derivatives, etc.) as well as bamipinacetate, buclizine, clemastine, clemizole, cromoglicinic acid, cyproheptadine, diflucorolonvalerate, dimetotiazine, diphenhydramine, diphenylpyraline, ephedrine, fluocinolane, histapyrrodine, isothipendyle, methadilazine, oxomemazine, paramethasone, prednilidene, theophilline, tolpropamine tritoqualine, etc. are used; amongst the preferred agents in this class are the substances characterized by their capacity to interfere (stimulate or suppress) the production of immunologically active substances, such as interleukines, interferones, leucotrienes, prostaglandines, etc. Amongst others, certain lipids and lipoids, such as phosphatidylcholines and diacylglycerols, or fatty acids and their esters, with chains containing several, preferably 3-6, most very frequently 3 or 4, double bonds, preferably of the n-3 type, are used for this purpose; the latter may also be hydroxygenated, branched or (partially) derivatized into ring structures.
- at least one substance with antiarrhythmic action, such as most of the cardiacs and beta-blockers, ajmaline, bupranolol, chinidine, digoxine derivatives, diltiazem, disopyramidedihydrogensulfate, erythromycine, disopyramide, gallopamil, ipratropiumbromide, lanatoside, lidocaine, lorcainide, orciprenalinesulfate, procaine amide, propafenone, sparteinesulfate, verapamil,

toliprolol.

- an antiarteriosclerotic, such as clofibrate.
- at least one substance belonging to the antiasthmatics and/or bronchospasmolytics, such as amiodarone, carbuterol, fenoterol, orciprenalin, sotalol, or theophylline-derivatives, as well as corticoids (such as beclomethasone, dexamethasone, hydrocortisone, prednisolone), frequently in combination with purines;
- at least one substance from the class of antibiotics, such as actinomycine, alamethicine, alexidine, 6-aminopenicillanic acid, moxycilline, amphotericine, ampicilline, anisomycine, antiamoebine, antimycine, aphidicoline, azidamfenicol, azidocilline, bacitracine, beclomethasone, benzathine, benzylpenicilline, bleomycine, bleomycine sulfate, calcium ionophor A23187, capreomycine, carbenicilline, cefacetril, cefaclor, cefamandole nafate, cefazoline, cefalexine, cefaloglycine, cefaloridine, cefalotine, cefapirine, cefazoline, cefoperazone, ceftriaxone, cefuroxim, cephalaxine, cephaloglycine, cephalothine, cephapirine, cerulenine, chloroamphenicol, chlorotetracycline, chloroamphenicol diacetate, ciclaciline, clindamycine, chloromadinone acetate, chloropheniramine, chromomycine A3, cinnarizine, ciprofloxacin, clotrimazole, cloxacilline, colistine methanesulfonate, cycloserine, deacetylanisomycine, demeclocycline, 4,4'-diaminodiphenyl sulfone, diaveridine, dicloxacilline, dihydrostreptomycine, dipyrindamol, doxorubicine, doxycycline, epicilline, erythromycine, erythromycine-stolate, erythromycinethylsuccinate, erythromycine stearate, ethambutol, flucloxacilline, fluocinolone acetonide, 5-fluorocytosine, filipine, formycine,

fumaramidomycine, furaltadone, fusidic acid, geneticine, gentamycine, gentamycine sulfate, gliotoxine, gfamicidine, griseofulvine, helvolic acid, hemolysine, hetacillin, kasugamycine, kanamycine (A), lasalocide, lincomycine, magnesidine, melphalane, metacycline, meticilline, mevinoline, micamycine, mithramycine, mithramycine A, mithramycine complex, mitomycine, minocycline, mycophenolic acid, myxothiazol, natamycine, nafcilline, neomycine, neomycine sulfate, 5-nitro-2-furaldehydesemicarbazone, novobiocine, nystatine, oleandomycine, oleandomycine phosphate, oxacihine, oxytetracycline, paromomycine, penicilline, pecilocine, pheneticilline, phenoxymethylpenicilline, phenyl amino-salicylate, phleomycine, pivampicilline, polymyxine B, propicilline, puromycine, puromycine aminonucleoside, puromycine aminonucleoside 5'-monophosphate, pyridinol carbamate, rolitetracycline, rifampicine, rifamycine B, rifamycine SV, spectinomycine, spiramycine, streptomycine, streptomycine sulfate, sulfabenzamide, sulfadimethoxine, sulfamethizol, sulfamethoxazol, tetracycline, thiamphenicol, tobramycine, troleandomycine, tunicamycine, tunicamycine A1-homologs, tunicamycine A2-homolog, valinomycine, vancomycine, vineomycine A1, virginiamycine M1, viomycine, xylostasine;

- at least one substance with an antidepressive or antipsychotic action, such as diverse monoaminoxidase-suppressors, tri- and tetracyclic antidepressives, etc. Very frequently used agents of this class are alprazolame, amitriptyline, chloropromazine, clomipramine, desipramine, dibenzepine, dimetacrine, dosulepine, doxepine, fluvoxaminhydrogenmaleate, imipramine, isocarboxazide, lofepramine, maprotiline, melitracene, mianserine, nialamide, noxiptiline,

nomifensine, nortriptyline, opipramol, oxypertine, oxytriptane, phenelzine, protriptyline, sulpiride, tranylcypromine, trosadone, tryptophane, vitoxazine, etc.

- at least one antidiabetic agent, such as acetohexamide, buformine, carbutamide, chloropropamide, glibenclamide, glibornuride, glymidine, metformine, phenformine, tolazamide, tolbutamide;
- at least one substance acting as an antidote, for example, against the heavy metal poisoning, poisoning with insecticides, against drugs, blood poisons, etc. A few examples are different chelators, amiphenazol obidoxim-chloride, D-penicillamine, tiopromine, etc.;
- at least one substance from the class of antiemetics: some of such suitable agents are alizapride, benzquinamide, betahistidine-derivatives, cyclizine, difenidol, dimenhydrinate, haloperidol, meclozine, metoclopramide, metopimazine, oxypendyl, perphenazine, pipamazine, piprinhydrinate, prochloroperazine, promazine, scopolamine, sulpiride, thiethylperazine, thioproperazine, triflupromazine, trimethobenzamide, etc., which are frequently used in combination with vitamins and/or antiallergics;
- at least one substance with an antiepileptic action, such as barbexaclone, barbiturate, beclamide, carbamazepine, chloroalhydrate, clonazepam, diazepam, ethosuximide, ethylphenacemide, lorazepam, mephenytoine, mesuximide, oxazolidine, phenaglycodol, phensuximide, phenytoine, primidone, succinimide-derivatives, sultiam, trimethadione, yalproinic acid, etc.; additives are commonly chosen from the classes of hypnotics and sedatives; an especially commonly used agent of this kind is

carbamazepine.

- at least one substance with antifibrinolytic activity, such as aminocaproic acid or tranexamic acid.
- at least one anticonvulsive agent, such as beclamide, carbamazepine, clomethiazole, clonazepam, methylphenobarbital, phenobarbital or sultiam;
- at least one substance which modifies choline concentration, by having an anticholinergic activity, for example. The following substances can be used, amongst others, as cholinergics: aubenoniumchloride, carbachol, cerulezide, dexpanthenol and stigmine-derivatives (such as distigminebromide, neostigminemethylsulfate, pyridostigmine-bromide); frequently used as anticholinergics are especially atropine, atropinmethonitrate, benactyzine, benzilonium-bromide, bevonium-methylsulfate, chlorobenzoxamine, ciclonium-bromide, clidinium-bromide, dicycloverine, diphemanil-methylsulfate, fempiverinium-bromide, glycopyrroniumbromide, isopropamide-iodide, mepenzolate-bromide, octatropine-methylbromide, oxyphencyclimine, oxyphenonium-bromide, pentapiperide, pipenzolate-bromide, piperidolate, pridinol, propanidide, tridihexethyl-iodide and trospiumchloride; cholinesterase inhibitors, such as ambenonium-chloride, demecarium-bromide, echothiopate-iodide, etc., are also useful for this purpose;
- at least one substance which can change, in the majority of cases diminish, the effect or concentration of histamine (antihistaminics). Preferred are hypoallergic carriers or hypoallergic edge active substances with n-3 (omega-3), less frequently with n-6 (omega-6), and mainly several, often 3-6 double bonds; such substances are

occasionally employed with hydroxy, more rarely methyl-, or oxo-side groups, or in an epoxy configuration; further suitable agents of this class are, among other substances, aethylenediamine, alimemazine, antazoline, bamipine, bromo-azine, bromo-pheniramine, buclizine, carbinoxamine, chlorocyclizine, chloropyramine, chlorophenamine, chlorophenoxamine, cimetidine, cinnarizine, clemastine, clemizol, colamine (such as diphenhydramine), cyclizine, dexbrompheniramine, dexchloropheniramine, difenidol, dimetindene, dimetotiazine, diphenhydramine, diphenylpyraline, dixyrazine, doxylamine, histapyrrodine, isothipendyl, mebhydroline, meclozine, medrylamine, mepyramine, methdilazine, pheniramine, piperacetazine, piprinhydrinate, pyrilamine (mepyramine), promethazine, propylamine, pyrrobutanine, thenalidine, tolpropamine, tripelennamine, triprolidine, etc.;

- at least one substance belonging to the class of antihypertonics, such as many alpha-receptor agonists, aldosterone-antagonists, angiotensine-converting-enzyme-blockers, antisymphaticotonics, beta-blockers, calcium-antagonists, diuretics, vasodilators, etc.; suitable agents for this purpose are for example alpenolol, atenolol, bendroflumethiazide, betanidine, butizide, chlorotalidone, clonidine, cycletanine, cyclopenthiiazide, debrisoquine, diazoxide, dihydralazine, dihydroergotaminmethanesulfonate, doxazinmesilate, guanethidine, guanoclor, guanoxane, hexamethonium-chloride, hydralazine, labetalol, mecanylanine, methyldopa, pargyline, phenoxybenzamine, prazosine, quinethazone, spironolactone, bescinnamine, reserpine, trichloromethiazide or vincamine;

- at least one substance which is an inhibitor of biological activity, such as actinomycine C1, alpha-

amanitine, ampicilline, aphidicoline, aprotinine, calmidazolium (R24571), calpaine-inhibitor I, calpaine-inhibitor II, castanospermine, chloroamphenicol, colcemide, cordycepine, cystatine, 2,3-dehydro-2-desoxy-n-acetyl-neuraminic acid, 1-desoxymannojirimycine-hydrochloride, 1-desoxynojirimycine, diacylglycerol-kinase-inhibitor, P1, P5-di(adenosine-5'-)-penta-phosphate, ebelactone A, ebelactone B, erythromycine, ethidiumbromide, N-hydroxyurea, hygromycine B, kanamycine sulfate, alpha2-macroglobuline, N-methyl-1-desoxynojirimycine, mitomycine C, myxothiazol, novobiocine, phalloidine, phenylmethanesulfonylfluoride, puromycine-dihydrochloride, rifampicine, staurosporine, streptomycine sulfate, streptozotocine, G-strophanthine, swainsonine, tetracycline-hydrochloride, trifluoperazine-dihydrochloride, tunicamycine, etc.; useful proteinase inhibitors are, for example, (4-amidinophenyl)-methanesulfonylfluoride (APMSF), antipaine-dihydrochloride, antithrombine III, alpha-1-antitrypsine, aprotinine, bestatine, calpaine-inhibitor I, calpaine-inhibitor II, L-1-chloro-3-(4-tosylamido)-7-amino-2-heptanone-hydrochloride (TLCK), L-1-chloro-3-(4-tosylamido)-4-phenyl-2-butanone (TPCK), chymostatine, cystatine, 3,4-dichlorisocoumarin, E 64, selastatinal, hirudin, kallikrein-inhibitor (aprotinine) L-leucinthiol, leupeptine, pepstatine, phenylmethanesulfonylfluoride (PMSF), phosphoramidone, TLCK (tosyl-lysine-chloromethylketone), TPCK (tosyl-phenylalanine-chloromethylketone), trypsine-inhibitors, etc.;

- at least one substance acting as an antihypotonic agent; quite frequently the corresponding drugs are from the classes of analeptics, cardiacs or corticoids. Suitable agents for this purpose are, for example, angiotensinamide, cardaminol, dobutamine, dopamine, etifelmine,

etilefrine, gepefrine, heptaminol, midodrine, oxedrine, etc., especially norfenefrine;

- at least one substance from the group of anticoagulants. Among other substances, some coumarin-derivatives are suitable for this purpose, as well as heparine and heparinoids, hirudine and related substances, dermatan-sulfate etc.; most frequently used agents of this class are acenocoumarin, anisindione, diphenadione, ethyl-biscoumacetate, heparine, hirudine, phenprocoumon, as well as warfarine;
- at least one substance from the class of antimycotics; well-suited examples of such agents include: amphotericine, bifanozol, buclosamide, chinoline-sulfate, chloromidazol, chlorophenesine, chloroquinaldol, clodantoine, cloxiquine, cyclopiroloxadine, dequaliniumchloride, dimazol, fenticlor, flucytosine, griseofulvine, ketoconazol, miconazol, natamycine, sulbentine, tioconazol, tolnaftate, etc.; particularly frequently, amphotericine, clotrimazol or nystatine are likely to be used for this purpose;
- at least one substance from the class of antimyasthenics, such as pyridostigmine-bromide;
- at least one substance which is active against morbus parkinson, such as amantadine, benserazide, benztropine, biperidene, cycrimine, levodopa, metixene, orphenadrine, phenglutarimide, pridinol, procyclidine, profenamine or trihexyphenidyl;
- at least one substance with an antiphlogistic activity, such as aescine, acetylsalicylic acid, alclofenac, aminophenazone, azapropazone, benzydamine, bumadizone,

chlorothenoxazine, diclofenac, flufenaminic acid, glafenine, ibuprofene, indometacine, kebuzone, mefenam acid, metiazic acid, mesalazine, mofebutazone, naproxene, niflumine acid, salts, such as Na-salt, noramido-pyriminium-methane-sulfonate, orgoteine, oxyphenbutazone, phenylbutazone, propyphenazone, pyridoxine, tolmetine, etc.; very suitable is, for example, ibuprofen; some of the agents commonly used as antiphlogistics also exhibit an antihistaminic or analgetic activity and belong to the classes of corticoids, vasoactiva, ophthalmics or otologics;

- at least one substance which is an antipyretic, such as acetylsalicylic acid, alclofenac, aminophenazone, benzydamine, bumadizone, chinine, chlorinethenoxazine, lactylphenetidine, meprob, paracetamol, phenacetine, propyphenazone or salicylamide;
- at least one substance with an antirheumatic activity, such as acetylsalicylic acid, benorilate, chloroquine, diclofenac, fenoprofene, flufenaminic acid, ibuprofene, kebuzone, lactylphenetidine, mefenamic acid, mofebutazone, naproxene, sodiumaurothiomalate, nifenazone, nifluminic acid, D-penicillamine and salicylamide. Edge active substances, carriers and/or agents, with a hypoallergic action, for example from the groups of analgetics, corticoids and glucocorticoids, enzymes or vitamins, etc., are preferred for this purpose, as well as antiphlogistics, such as quinine, nicotinic acid-, nonylic acid-, or salicylic acid-derivatives, meprobamate, etc.;
- at least one antiseptic such as acriflaviniumchloride, cetalkonium-chloride, cetylpyridinium-chloride, chlorohexidine, chloroquinaldol, dequaliniumchloride,

domiphen-bromide, ethacridine, hexetidine, merbromine, nitrofurazone, oxyquinol, phanquinone, phenazopyridine or phenylmercuriborate, as well as fatty acids with an uneven number of carbon atoms;

- at least one respiratory analeptic or respiration stimulant, such as amiphenazol, ascorbic acid, caffeine, cropropamide, crotethamide, etamivane, ephedrine, fominobene, nicethamide; or aminophenazol and doxaprame, for example;
- at least one broncholytic, such as bamifylline, beclometasone, dexometasone (e.g. in dexometasone-21-isonicotinate), diprophylline, ephedrine (e.g. in ephedrinehydrogentartrate), fenoterol, hexoprenaline, ipratropium-bromide, iso-eterine, isoprenaline, orciprenaline, protocylol, proxyphylline, reproterol, salbutamol, terbutaline, tetroquinol, theophylline, etc.; and biological extracts, for example from anis, eucalyptus, thyme, etc.;
- one cardiostimulant, especially aminophylline, benfurodilhemisuccinate, etofylline, heptaminol, protheobromine or proxyphylline;
- at least one substance from the class of chemotherapeutic agents, for example, acediasulfone, acriflavinium-chloride, ambazone, dapson, dibrompropamide, furazolidone, hydroxymethylnitrofurantoin, idoxuridine, mafenide and sulfateolamide, mepacrine, metronidazole, nalidixic acid, nifuratel, nifuroxazide, nifurazone, nifurtimox, ninorazole, nitrofurantoin, oxolinic acid, pentamidine, phenazopyridine, phthalylsulfatehiazole, pyrimethamine, salazosulfapyridine, sulfacarbamide, sulfacetamide, sulfachloropyridazine, sulfadiazine,

sulfadiazine, sulfadimethoxine, sulfaethidol, sulfafurazol, sulfaguanidine, sulfaguanol, sulfamethizol, sulfamethoxazol and cotrimoxazol, sulfamethoxydiazine, sulfamethoxypyridazine, sulfamoxol, sulfanilamide, sulfaperine, sulfaphenazol, sulfathiazol, sulfisomidine, tinidazol, trimethoprim, etc.;

- at least one substance from the class of coronary dilators, such as bamifylline, benziodarone, carbochromes, dilazep, dipyridamol, etafenone, fendiline, hexobendine, imolamine, lidoflazine, nifedipine, oxyfedrine, pentaerythrityltetranitrate, perhexiline, prenylamine, propatylnitrate, racefemine, trolnitrate, verapamil, visnadine, etc.;
- at least one cytostatic, for example, from the group of alkylating agents, antibiotics, platinum compounds, hormones and their inhibitors, interferones, etc.; very frequently used substances of this kind are:
acliarubicine, azathioprine, bleomycine, busulfane, calciumfolinate, carboplatinum, carmustine, chloroambucil, cis-platinum, cyclophosphamide, cytarabine, daunorubicine, epirubicine, fluorouracil, fosfestrol, hydroxycarbamide, ifosfamide, lomustine, melphalan, mercaptopurine, methotrexate, mitomycin C, mitopodizide, mitramycin, nimustine, pipobromane, prednimustine, procarbazine, testolactone, theosulfane, thiotepa, tioguanine, triaziquone, trofosfamide, vincristine, vindesine, vinblastine, zorubicine, etc.;
- an intestinal antiseptic, such as broxyquinoline, clioquinol, dihydroxyquinoline, halquinol, etc.;
- at least one diuretic, such as acetazolamide, aminophylline, bendroflumethiazide, bumetanide, butizide,

chloroazanile, chloromerodrine, chlorothiazide, chloro-
talidone, clopamide, clorexolone, cyclopenthiiazide,
cyclothiazide, etacrynic acid, furosemide, hydrochloro-
thiazide, hydroflumethiazide, mefruside, methazolamide,
paraflutizide, polythiazide, quinethazone, spirono-
lactone, triamterene, trichloromethiazide, xipamide,
etc.;

- at least one ganglion blocker, such as gallamintri-
ethiodide, hexamethonium-chloride, mecamlamine, etc.;
- at least one substance for the therapy of arthritis,
preferably analgetics or for example allopurinol,
benzbromarone, colchicine, benziodarone, probenecide,
sulfipyrazone, tenoxicam, etc.; in very many cases
allopurinol;
- at least one glucocorticoid, such as beclomethason,
betamethason, clocortolone, cloprednol, cortison, dexamethason (e.g. as a dexamethasonephosphate), fludrocortison, fludroxycortide, flumetason, fluocinolon-acetonide, fluocinonide, fluocortolon (e.g. as a fluocortoloncapronate or fluocortolontrimethylacetate), fluorometholon, fluprednidenacetate, hydrocortison (also as a hydrocortison-21-acetate, hydrocortison-21-phosphate, etc.), paramethason, prednisolon (e.g. in the form of methylprednisolon, prednisolon-21-phosphate, prednisolon-21-sulfobenzoate, etc.), prednison, prednyliden, pregnenolon, triamcinolon, triamcinolonacetonide, etc.;
- at least one agent with a putative anti-flew action, such as moroxydine;
- at least one haemostatic, such as adrenalon, ascorbic

acid, butanol, carbazochrome, etamsylate, protamine, samatostatine etc.; thyroidal hormones and vitamins can be employed for this purpose as well;

- at least one hypnotic, from the class of barbiturates, benzodiazepines, bromo-compounds, ureids, etc., for example; quite commonly applied for this purpose are, e.g. acecarbromal, alimemazintartrate allobarbital, amobarbital, aprobarbital, barbital, bromo-isoval, brotizolam, carbromal, chloroalhydrate, chloroalodol, chlorobutanol, clomethiazol, cyclobarbital, diazepam, diphenhydramine, doxylamine, estazolam, ethchlorvynol, ethinamate, etomidate, flurazepam, glutethimide, heptabarb, hexobarbital, lormetazepam, malperol, meclozine, medozine, methaqualon, methyprylon, midazolam, nitrazepam, oxazepam, pentobarbital, phenobarbital, promethazine, propallylonal, pyrithyldion, secbuta-barbital, secobarbital, scopolamine, temazepam, triazolam, vinylbital, etc.; various extracts from balm-mint, valerian, and passiflora are also used;
- at least one immunoglobuline, from the IgA, IgE, IgD, IgG, IgM classes or an immunoglobuline fragment, such as a Fab- or Fab2-fragment, or the corresponding variable or hypervariable region, if required in combination with other agents and/or chemically, biochemically or genetically manipulated;

An immunoglobuline can be of the IgA, IgD and IgE, IgG (e.g. Ig G1, Ig G2, Ig G3, Ig G4) or IgM type. In the context of this application, any chemical or biochemical derivative of any immunoglobuline (Ig) is considered useful, for example, an Ig G-gamma chain, an Ig G-F(ab')₂ fragment, an Ig G-F(ab) fragment, an Ig G-Fc fragment, an Ig-kappa chain, a light chain of Ig-s (e.g. a kappa and

lambda chain), but also even smaller immunoglobuline fragments, such as the variable or hypervariable regions, or artificial modifications of any of these substances.

- at least one substance with an immunostimulating activity, with an immunosuppressive potency, with a capability to give rise to the production of immunoglobulines or other immunologically active substances (endotoxines, cytokines, lymphokines, prostaglandines, leucotrienes, other immuno modulators or biological messengers), including vaccines. Antibodies against any of these substances can also be used; preferred are immunotransfersomes with or without endotoxines, cytokines, prostaglandines, leucotrienes, with other immunomodulators, immunologically active cellular or molecular fragments, as well as corresponding antagonists, derivatives or precursors; particularly preferred compounds are lipid A and other glycolipids, muraminic acid derivatives, trehalose derivatives, phythaemagglutinines, lectins, polyinosine, polycytidylic acid (poli I:C), dimepranol-4-acetamidobenzoate, erythropoietin, 'granulocyte-macrophage colony stimulating factor' (GM-CSF), interleukine I and II, III and VI, interferon alpha, beta and/or gamma, leucotriene A, B, C, D, E and F, propandiamine, prostaglandine A, B, C, D, E, F, and I (prostacycline), tumor necrosis factor-alpha (TNF-alpha), thromboxan B, as well as immunoglobulines of types IgA, IgE, IgD, IgG, IgM; furthermore, suitable tissue and plant extracts, their chemical, biochemical or biological derivatives or replacements, their parts, such as characteristic peptide chains, etc.; as immunosuppressives, ganciclovir, azathiiprin, cyclosporin, FK 506 etc. are frequently used;

- at least one contraceptive agent, such as medroxyprogesteronacetate, lynesterol, lvonorgestrel, norethisteron, etc.;
- at least one circulation analeptic, such as cafedrin, etamivan, etilefrin, norfenefrin, pholedrin, theodrenalin, etc.;
- at least one drug for the therapy of liver diseases, such as orazamide, silymarin, or tiopromin;
- at least one substance with a light-protective function, such as mexenone;
- at least one antimalaria agent, such as amodiaquin, hydroxychloroquin or mepacrin;
- at least one substance for migraine or schizophrenia treatment, such as certain analeptics, beta-blockers, clonidin, dimetotiazine, ergotamine, lisurid (hydrogen maleate), methysergide, pizotifen, propranolol, proxibarbal, etc. Even more suitable are the serotonin antagonists or the blockers of serotonin receptors, such as 5-HT₁, 5-HT₂ or 5-HT₃; well suited for use according to this invention are also the receptor blockers AH21467 (Glaxo), AH25086 (Glaxo), GR43175 (Glaxo), GR38032 (Glaxo, = ondansetron), 5-hydroxytryptamine, ketanserine, methiothepin, alpha-methyl-5HT, 2-methyl-5HT, etc.;
- at least one mineral corticoid, such as aldosterone, fludrocortison, desoxycortonacetate, corresponding derivatives, etc.;
- at least one morphine antagonist (such as amiphenazol, lealvallorphan, nalorphine) or some substance with

morphine-like properties such as casomorphine, cyclo(leu-gly), dermorphine, met-enkephaline, methorphanide (tyr-gly-gly-phe-met-arg-arg-val), morphiceptine, morphine modulating neuropeptide (ala-gly-glu-gly-leu-ser-ser-pro-phe-trp-ser-leu-ala-ala-pro-gln-arg-phe-NH₂) etc.;

- at least one muscle relaxant, which frequently belongs to the groups of competitively or depolarising curare-agents, myotonolytics or analgetics; suitable substances with the desired effect are, among other materials, acetylsalicylic acid, alcuronium-chloride, azapropazon, atracuriumbesilate, baclofen, carisoprodol, quinine derivatives, chloromezanol, chlorophenesincarbamate, chlorozoxazon, dantrolen, decamethoniumbromide, dimethyltubocurariniumchloride, fenylamidol, gallamintriethiodide, guaiphensine, hexafluorenium-bromide, hexacarbacholinbromide, memantin, mephensin, meprobamate, metamisol, metaxalon, methocarbamol, orphenadrin, paracetamol, phenazon, phenprobamate, suxamethoniumchloride, tetrazepam, tizanidin, tubocurarinchloride, tybamate, etc.;
- at least one narcotic, such as alfentanil, codeine, droperidol, etomidate, fentanyl, flunitrazepam, hydroxybutiric acid, ketamine, methohexital, midazolam, thebacon, thiamylal, thiopental, etc., as well as corresponding derivatives;
- at least one substance with a neurotherapeutic activity, such as anaesthetics and vitamins, atropine-derivatives, benfotiamine, choline-derivatives, caffeine, cyanocobalamine, alpha-liponic acid, mepivacaine, phenobarbital, scopolamine, thiaminchloride hydrochloride, etc., and, most notably, procaine;

- at least one neuroleptic, e.g. butyrophenon-derivatives, phenotiazin-derivatives, tricyclic neuroleptics, as well as acetophenazine, benperidol, butaperazine, carfenazine, chloropromazine, chloroprothixen, clopenthixol, clozapine, dixyrazine, droperidol, fluanison, flupentixol, fluphenazine, fluspirilen, haloperidol, homofenazine, levomepromazine, melperon, moperon, oxipertin, pecazine, penfluridol, periciazine, perphenazine, pimozide, pipamperon, piperacetazine, profenamine, promazine, prothipendyl, sulforidazine, thiopropazate, thioproperazine, thioridazine, tiotixen, trifluoperazine, trifluoperidol, triflupromazine, etc.; in particular, haloperidol and sulperide are often used for this purpose;
- at least one neurotransmitter or one of its antagonists; preferably, acetylcholine, adrenaline, curare (and, e.g. its antagonist edrophonium-chloride), dopamine, ephedrine, noradrenaline, serotonin, strychnine, vasotonine, tubocurarine, yohimbine, etc. are used;
- at least one ophthalmic, in many cases from the groups of anaesthetics, antibiotics, corticoids, eye-tonics, chemotherapeutics, glaucoma agents, virustatics, antiallergics, vasodilatators, or vitamins;
- at least one parasympathicomimetic (e.g. bethanechol-chloride, carbachol, demecarium-bromide, distigmin-bromide, pyridostigmin-bromide, scopolamine) or at least one parasympathicolytic (such as benzatropine, methscopolamine-bromide, pilocarpine or tropicamide);
- at least one agent for the therapy of psoriasis and/or neurodermitis; particularly well suited for this purpose are carrier substances with a hypoallergic action or the corresponding edge active compounds, with n-3 (omega 3),

less frequently with n-6 (omega 6), mainly with multiple, often 3-6, double bonds and/or hydroxy, more seldom methyl-, or oxo-side groups; these can also appear as side chains on further agent molecules; side groups on the 15th carbon atom are particularly efficient; as additives, amongst other substances, antimycotics, cytostatics, immunosuppressants or antibiotics can be used;

- at least one agent for the dilatation of the iris (mydriatic), such as atropine, atropinmethonitrate, cyclopentolate, pholedrine, scopolamine or tropicamide;
- at least one substance with a psychostimulating action; well suited for this purpose are, for example, amphetaminil, fencamfamine, fenetylline, meclofenoxate, methamphetamine, methylphenidate, pemoline, phendimetrazine, phenmetrazine, prolintane or viloxazine;
- at least one rhinologic, such as buphenine, cafaminol, carbinoxamide, chlorophenamin, chlorotenoxyzine, clemastine, dextromethorpane, etilefrine, naphazoline, norephedrine, oxymetazoline, phenylaprhine, piprinydrinate, pseudoephedrine, salicylamide, tramazoline, triprolidine, xylometazoline, etc.; from biological sources especially the radix gentiane extract;
- at least one somnifacient (such as sleep-inducing peptide (trp-ala-gly-gly-aspartic-acid-ser-gly-glu)), or a corresponding antagonist (such as bemegride);
- at least one sedative or tranquilizer, as the former, for example, acecarbromal, alimemazine, allobarbitol, aprobarbitol, benzoctamine, benzodiazepine-derivatives,

bromo-isoval, carbromal, chloropromazine, clomethiazol, diphenyl-methane-derivatives, estazolam, fenetylline, homofenazine, mebutamate, mesoridazine, methylpentynol, methylphenobarbital, molindone, oxomemazine, perazine, phenobarbital, promethazine, prothipendyl, scopolamine, secbutabarbital, trimetozine, etc.; as a tranquilizer, for example, azacyclonol, benactyzin, benzoctamine, benzquinamide, bromo-azepam, chlorodiazepoxide, chlorophenesincarbonate, cloxazolam, diazepam, dipotassium-chloroazepate, doxepine, estazolam, hydroxyzine, lorazepam, medazepam, meprobamate, molindone, oxazepam, phenaglycodol, phenprobamate, prazepam, prochloroperazine, rescinnamine, reserpine or tybamate; drugs, such as distraneurine, hydantoin-derivatives, malonyl uric acid-derivatives (barbiturates), oxazolidine-derivatives, scopolamine, valepotriate, succinimide-derivatives, or hypnotics (e.g. diureides (such as barbiturates)), methaqualon, meprobromate, monoureides (such as carbromal), nitrazepam, or piperidin-dione, can be used for this purpose; amongst other substances, certain thymoleptics, such as librium or tofranil, can be used as antidepressants;

- at least one substance from the class of spasmolytics, e.g. adiphenine, alverine, ambicetamide, aminopromazine, atropine, atropine methonitrate, azintamide, bencyclane, benzarone, bevonium-methylsulfate, bietamiverine, butetamate, butylscopolammoniumbromide, camylofine, carzenide, chlorodiazepoxide, cionium-bromide, cyclandelate, cyclopentolate, dicycloverine, diisopromine, dimoxyline, diphemanil-methylsulfate, ethaverine, ethenzamide, fencarbamide, fenpipramide, fenpivennum-bromide, gefarnate, glycopyrroniumbromide, hexahydroadiphenin, hexocycliummethylsulfate, hymecromon,

isometheptene, isopropamidiodide, levomethadone, mebeverine, metamizon, methscopolamine-bromide, metixen, octatropine-methylbromide, oxazepam, oxybutin, oxyphenonium-bromide, papaverine, paracetamol, pentapiperide, penthienate-methobromide, pethidine, pipenzolate-bromide, piperidolate, pipoxolane, propanthelin-bromide, propylphenazon, propyromazine-bromide, racefemine, scopolamine, sulpiride, tiemonium-iodide, tridihexethyliodide, tropenzilinbromide, tropinbenzilate, trospiumchloride, valetamatbromide, etc.; furthermore, belladonna alkaloids, papaverine and its derivatives, etc.;

- at least one sympathicolytic, e.g. azapetine or phentolamine;
- at least one sympathicomimetic, e.g. bamethane, buphenine, cyclopentamine, dopamine, L-(-)-ephedrine, epinephrine, etilefrine, heptaminol, isoetarine, metaraminol, methamphetamine, methoxamine, norfenefrine, phenylpropanolamine, pholedrine, propylhexedrine, protokylol or synephrine;
- at least one tuberculostatic, such as an antibiotic, p-aminosalicylic acid, capreomycine, cycloserine, dapson, ethambutol, glyconiazide, iproniazide, isoniazide, nicotinamide, protionamide, pyrarinamide, pyrodoxine, terizidone, etc., and, particularly preferred thereof, ethambitol and isoniazide;
- at least one urologic, e.g. a bladder tension modifying agent (such as cholinecitrate, distigminebromide, yohimbine), a corresponding antiinfection agents (antibiotics, chemotherapeutics, or nitrofurantoid-, chinolone-, or sulfonamide-derivative); furthermore,

adipinic acid, methionine, methenamine-derivatives, etc.;

- at least one substance with a vasoconstricting action; often, adrenalone, epinephrine, felypressine, methoxamine, naphazoline, oxymetazoline, tetrazyoline, tramazoline or xylometazoline are used for this purpose;
- at least one substance which is a vasodilatator, such as e.g. azapetine, banethane, bencyclane, benfurodil-hemisuccinate, buphenine, butalamine, cinnarizine, diprophylline, hexyltheobromine, ifenprodil, isoxsuprine, moxislyte, naftidrofuryl, nicotinylalcohol, papaverine, phenoxybenzamine, piribedil, primaperone, tolazoline, trimetazidine, vincamine or xantinol-nicotinate;
- at least one veins agent, e.g. aescine, benzarone, calcium-dobesilate, dihydroergotaminemesilate, diosmine, hyhydroxyethylrutoside, pignogenol, rutoside-aesinate, tribenoside, troxerutine, etc.;
- at least one virustatic, e.g. one immunostimulating agent, and/or an additional drug, such as as moroxydine or tromantadine, which may stimulate action of the immunostimulator;
- one agent for the treatment of wounds; for example, dexpanthenol, growth stimulating factors, enzymes or hormones, especially in combination with carriers which contain essential substances; povidon-iodide, fatty acids which are not straight, cetylpyridiniumchloride, chinoline-derivatives of known antibiotics and analgetics are useful;
- at least one substance with a toxic action or a toxin; common toxins from plant or microbial sources in

particular 15-acetoxyscirpenol, 3-acetyldeoxynivalenol, 3-alpha-acetyldiacetoxyscirpenol, acetyl T-2 toxin, aflatoxicol I, aflatoxicol II, aflatoxin B1, aflatoxin B2, aflatoxin B2-alpha, aflatoxin G1, aflatoxin G2, aflatoxin G2-alpha, aflatoxin M1, aflatoxin M2, aflatoxin P1, aflatoxin Q1, alternariol-monomethyl ether, aurovertin B, botulinum toxin D, cholera toxin, citreoviridin, citrinin, cyclopiazonic acid, cytochalasin A, cytochalasin B, cytochalasin C, cyrochalasin D, cytochalasin, cytochalasin H, cytochalasin J, deoxynivalenol, diacetoxyscirpenol, 4,15-diacetylverrucarol, dihydrocytochalasin B, enterotoxin STA, fusarenon X, iso T-2 toxin, O- methylsterigmatocystin, moniliformin, monoacetoxyscirpenol, neosolaniol, ochratoxin A, patulin, penicilinic acid, pertussis toxin, picrotoxin, PR-toxin, prymnesin, radicinin, roridin A, rubratoxin B, scirpentriol, secalononic acid D, staphylococcal enterotoxin B, sterigmatocystin, streptolysin O, streptolysin S, tentoxin, tetrahydrodeoxyaflatoxin B1, toxin A, toxin II, HT-2 toxin, T-2-tetraol, T-2 toxin, trichothecin, trichothecolon, T-2 triol, verrucaric acid, verrucarol, vomitoxin, zearalenol and zearalenone.

- at least one substance which affects growth in humans or animals, such as basic fibroblast growth factor (BFGF), endothelial cell growth factor (ECGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin, insulin-like growth factor I (LGF I), insulin-like growth factor II (LGF II), nerves-growth factor-beta (NGF-beta), nerves growth-factor 2,5s (NGF 2,5s), nerves growth-factor 7s (NGF 7s), platelet-derived growth factor (PDGF), etc.;
- a carrier and/or agent which creates a protective layer on and/or in a barrier, such as skin, against poison,

light UV-, gamma- or other radiation; against detrimental biological agents such as viruses, bacteria, toxins, etc.; carrier components and/or agents can hamper the detrimental action by chemical, biochemical, or biological means or else may prevent or diminish the penetration of such adversary agents;

- at least one fungicide, herbicide, pesticide, or insecticide;
- at least one plant hormone, e.g. abscisic acid, abscisic acid-methylester, 3-acetyl-4-thiazolidine-carboxyl acid, 1-allyl-1-(3,7-dimethyloctyl)-piperidinium bromide, 6-benzylaminopurine, 6-benzylaminopurine 9-(beta-glucoside), butanedio acid mono(2,2-dimethyl hydrazide), chlorocholine chloride, 2-chloroethyl-tris-(2'-methoxyethoxy)silane, 2-(o-chlorineophenoxy)-2-methylpropionic acid, 2-(p-chlorophenoxy)-2-methylpropionic acid, 2-(o-chlorophenoxypropionic acid, 2-(m-chlorophenoxy)propionic acid, clofibrinic acid, colchicine, o-coumarinic acid, p-coumarinic acid, cycloheximide, alpha,beta-dichloroisobutiric acid, 2-(2,4-dichlorophenoxy)propanoic acid, 2,3-dihydro-5,6-diphenyl 1,4-oxathiine, dihydrozeatine, 6-(gamma,gamma-dimethylallylamino)purino riboside, 3-(2-[3,5-dimethyl-2-oxocyclohexyl-2-hydroxyethyl])-glutarimide, trans-2-dodecenedioic acid, ethyl-8-chloro-1-indazol-3-yl-acetate, N6-furfuryladenosine, 6-furfurylamino-purineriboside, gibberellic acid methylester, gibberellin A3-acetate, gibberellin A1 methylester, gibberellin A4 methylester, gibberellin A5 methylester, gibberellin A7 methylester, gibberellin A9 methylester, gibberellin A3 methylester 3,13-diacetate gibberinic acid, allo-gibberinic acid, gibberinic acid methylester, glyoxim, 22(s),23(s)-homobrassinolide, 9-hydroxyfluorene 9-

carboxylate, indol-3-acetic acid, indol-3-acetic acid ethylester, indol-3-propanoic acid, N6-(2-isopentenyl)adenine, N6-(2-isopentenyl)adenosine, 2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine-carboxylat methylchloride, kinetinglucoside, kinetinriboside, melissylalcohol, 1-methyladenine, methyl 2-chloro-9-hydroxy-fluorene-9-carboxylate, methyl 3,6-dichloro-o-anisate, 6-methylmercaptapurine, 1-naphthylacetamide, nonanoic acid methylester, 6-piperidino-1-purine, n-triacontanol, (-)-xanthoxine, zeatine glucosides, etc.;

- at least one pheromone or one pheromone-like substance, such as (-)-bornyl acetate, trans-5-decenol, cis-5-decenyl acetate, trans-5-decenyl acetate, 2,6-dichlorophenol, 1,7-dioxaspiro[5.5]undecane, trans-8,trans-10-dodecadienol ([E,E]-8,10-DDDOL), trans-7, cis-9-dodecadienyl acetate ([E,Z]-7,9-DDDA), trans-8, trans-10-dodecadienyl acetate ([E,E]-8,10-DDDA), cis-7-dodecen-1-ol (Z-7-DDOL), trans-10-dodecenol, cis-7-dodecenyl acetate (Z-7-DDA), cis-8-dodecenyl acetate, trans-8-dodecenyl acetate, 11-dodecenyl acetate, cis-7,8-epoxy-2-methyl-octadecane, cis-9-heneicosene, cis-7,cis-11-hexadecadienylacetate ([Z,Z]-7,11-HDDA), cis-7,trans-11-hexadecadienyl acetate ([Z,E]-7,11-HDDA), cis-9-hexadecenal (Z-9-HDAL), cis-11-hexadecenal (Z-11-HDAL), cis-11-hexadecenol (Z-11-HDOL), cis-11-hexadecenyl acetate (Z-11-HDA), trans-2-hexenyl acetate, cis-7-tetradecenal (Z-7-TDAL), cis-9-tetradecenol (Myristoleyl alcohol; Z-9-TDOL), cis-7-tetradecenol (Z-7-TDOL), cis-11-tetradecenol, cis-7-tetradecenyl acetate (Z-7-TDA), cis-9-tetradecenyl acetate (Myristoleyl acetate; Z-9-TDA), cis-11-tetradecenyl acetate (Z-11-TDA), trans-11-tetradecenyl acetate (E-11-TDA), cis-9-tetradecenyl formate

(Myristoleyl formate; Z-9-TDF), isoamyl acetate (acetic acid 3-methylbutyl ester), 2-methyl-3-buten-2-ol, 3-methyl-2-cyclohexen-1-ol, cis-14-methyl-8-hexadecenal, cis-2-methyl-7-octadecene, 4-methylpyrrole-2-carboxylic acid methyl ester (Methyl 4-methylpyrrole 2-carboxylate) cis-13-octadecenal 13-octadecyn-1-ol, 2-(phenyl)ethyl propionate (phenylethanol propanoate), propyl cyclohexylacetate, cis-9,trans-11-tetradecadienol ([Z,E]-9,11-TDDOL), cis-9,trans-11-tetradecadienyl acetate ([Z,E]-9,11-TDDA), cis-9,trans-12-tetradecadienyl acetate ([Z,E]-9,12-TDDA), trichloroacetic acid esters, cis-9-tricosene, undecanal, etc.;

- at least one pigment or one colouring substance;
- at least one carbohydrate;

A carbohydrate, normally, has a basic formula $C_x(H_2O)_y$, e.g. in sugar, starch, cellulose, and, moreover, can be derivatised in many different ways.

A monomeric carbohydrate residue is, for example, a natural monosaccharide residue, which in many cases is an adduct of a pentose or a hexose in aldose or ketose form which, in principle, can adopt L- or D-configurations. Owing to the space constraints and due to their greater biological relevance, only the latter will be referred to in the following.

An aldose with five carbon atoms (aldo-pentose, or simply pentose) is for example D-arabinose, D-lyxose, D-ribose or D-xylose.

A ketose with five carbon atoms (keto-pentose) is e.g. D-ribulose or D-xylulose.

An aldose with six carbon atoms (aldo-hexose, or simply hexose) is e.g. D-allose, D-altrose, D-galactose, D-glucose, D-mannose or D-talose. A ketose with six carbon atoms (or simply keto-hexose) is e.g. D-fructose, D-psicose, D-sorbose or D-tagatose.

A hexose, very frequently, exists in a cyclic form, as a pyranose (aldose), for example; alpha- or beta-D-glucopyranose are two typical examples for this.

Another type of hexose is furanose, e.g. in an alpha- or beta-D-fructose. The pyranosyl residue is particularly preferably conjugated to a hydroxy group, the latter then being located in 1- or 6-positions; the furanosyl residue is preferably conjugated to the corresponding groups in positions 1- or 5-.

A carbohydrate residue, moreover, can be a natural disaccharide residue, e.g. a disaccharide residue consisting of two hexoses. Such a disaccharide residue arises, for example, through condensation of two aldoses, e.g. D-galactose or D-glucose, or one aldose, e.g. D-glucose and one ketose, e.g. fructose; disaccharides formed from two aldoses, such as lactose or maltose, are preferably conjugated to the phosphatidyl group through the hydroxy group, which is located in position 6- of the corresponding pyranosyl residue. A disaccharide formed from an aldose and a ketose, such as saccharose, is preferably conjugated through a hydroxyl-group in position 6- of the pyranosyl residue or in position 1- of the furanosyl residue.

A carbohydrate residue, moreover, is any derivatised mono-, di- or oligosaccharide residue, in which, for example, an aldehyde group and/or one or two terminal

hydroxy groups are oxidized to carboxy groups, e.g. in a D-glucar-, D-glucon- or D-glucuronic acid residue, all such residues being normally in the form of cyclic lactone residues. The aldehyde- or keto-groups in a derivatised mono- or disaccharide residue, moreover, can be reduced to hydroxy groups, e.g. in inositol, sorbitol or D-mannitol. Furthermore, individual hydroxy groups can be replaced by hydrogen atoms, e.g. in desoxysugars, such as 2-desoxy-D-ribose, L-fucose or L-rhamnose, or through amino groups, e.g. in aminosugars, such as D-galactosamine or D-glucosamine.

A carbohydrate can result from a cleaving action, starting with one of the mentioned mono- or disaccharides, by a strong oxidation agent, such as periodic acid. Amongst the biologically most important or most active carbohydrates are e.g. 2-acetamido-N-(epsilon-amino-caproyl)-2-deoxy-beta-glucopyranosylamine, 2-acetamido-1-amino-1,2-dideoxy-beta-glucopyranose, 2-acetamido-1-beta-(aspartamido)-1,2-dideoxyglucose, 2-acetamido-4,6-o-benzyliden-2-deoxy-beta-glucopyranose, 2-acetamido-2-deoxyallose, 3-acetamido-3-deoxyallose, 2-acetamido-2-deoxy-3-o-(beta-galactopyranosyl)-galactopyranose, 2-acetamido-2-deoxy-4-o-([4-o-beta-galactopyranosyl-beta-galactopyranosyl]-beta-galactopyranosyl)-glucopyranose, 2-acetamido-2-deoxy-3-o-(beta-galactopyranosyl)-alpha-glucopyranose, 6-o-(2-acetamido-2-deoxy-4-o-[beta-galactopyranosyl]-beta-glucopyranosyl)-galactopyranose, 4-o-acetamido-2-deoxy-6-o-(beta-galacto-4-o-(6-o-[2-acetamido-2-deoxy-beta-glucopyranosyl]-beta-galactopyranosyl) glucopyranose, 2-acetamido-2-deoxygalactose, 2-acetamido-2-deoxyglucose, 3-acetamido-3-deoxyglucose pyranose, 6-o-(2-acetamido-2-deoxy-beta-glucopyranosyl)-galactopyranose, 2-acetamido-2-deoxy-1-thio-beta-glucopyranose 3,4,6-

triacetate, acetopyruvic acid, N-acetylchondrosamine, N-acetylgalactosamine, N-acetylglucosamine, N-acetyl-alpha-glucosamine 1-phosphate, N-acetylglucosamine 6-phosphate, N-acetylglucosamine 3-sulfate, N-acetylglucosamine 6-sulfate, N-acetylheparine, N-acetyllactosamine, N-acetyl-beta-mannosamine, N-acetylneuraminic acid, N-acetylneuramine-lactose, 1-o-acetyl-2,3,5-tri-o-benzoyl-beta-ribofuranose, trans-aconic acid, adenine-9-beta-arabinofuranoside, adenosine 5'-diphospho-glucose, adenosine 5'-diphosphomannose, adonite, adonitol, adonose, agar, algin, alginic acid, beta-allose, alpha glycerophosphate, alpha ketoglutaric acid, altrose, (-)-altrose, p-aminobenzyl-1-thio-2-acetamido-2-deoxy-beta-glucopyranoside, N-epsilon-aminocaproyl-beta-fucopyranosylamine, N-epsilon-aminocaproyl-alpha-galactopyranosylamine, 2-amino-2-deoxygalactopyranose, 6-amino-6-deoxyglucopyranose, 1-amino-1-deoxy-beta-glucose, 6-aminoethyl-N-acetyl-beta-thioglucoaminide, 6-aminoethyl-1-thio-beta-galactopyranoside, 5-aminoimidazole-4-carboxamidoxime-1-beta-ribofuranosyl 3':5'-cyclo-monophosphate, delta-aminolevulinic acid, p-aminophenyl-2-acetamido-2-deoxy-beta-glucopyranoside, p-aminophenyl-2-acetamido-2-deoxy-1-thio-beta-glucopyranoside, p-aminophenyl-alpha-fucopyranoside, p-aminophenyl-alpha-galactopyranoside, p-aminophenyl-beta-galactopyranoside, p-aminophenyl-alpha-glucopyranoside, p-aminophenyl-beta-glucopyranoside, c-aminophenyl-beta-glucuronide, p-aminophenyl-1-thio-beta-glucuronide, p-aminophenyl-beta-lactopyranoside, p-aminophenyl-alpha-mannopyranoside, p-aminophenyl-beta-thiofucopyranoside, p-aminophenyl-1-thio-beta-galactopyranoside, p-aminophenyl-1-thio-beta-glucopyranoside, p-aminophenyl-1-thio-beta-xylopyranoside, p-aminophenyl-beta-xylopyranoside, 5-amino-1-(beta-ribofuranosyl)imidazole 4-carboxamide, amygdaline, n-amyl-beta-glucopyranoside, amylopectine, amylose, apigenine 7-

o-hesperidoside, arabinitol, arabinocytidine, 9-beta-arabinofuranosyladenine, 1-beta-arabinofuranosylcytosin, arabinose, arabinose 5-phosphate, arabinosylcytosine, arabite, arabitol, arbutine, atp-ribose, atractyloside, aurothioglucose, n-butyl 4-o-beta-galactopyranosyl-beta-glucopyranoside, calcium gluconate, calcium heptagluconate, carboxyatractyloside, carboxymethylamylose, carboxymethylcellulose, carboxyethylthioethyl-2-acetamido-2-deoxy-4-o-beta-galactopyransol-beta-glucopyranoside, carboxyethylthioethyl 4-o-(4-o-[6-o-alpha-glucopyranosyl-alpha-glucopyranosyl]-alpha-glucopyranosyl)-beta-glucopyranoside, 4-o-(4-o-[6-o-beta-D-galactopyranosyl-beta-D-galactopyranosyl]-D-glucopyranose, carrageenan, D(+)cellobiose, D(+)cellopentaose, D(+)cellotetraose, D(+)cellotriose, cellulose, cellulose caprate, cellulose carbonate, chitin, chitobiose, chitosan, chitotriose, alpha-chloroalose, beta-chloroalose, 6-chloro-6-deoxy-alpha-glucopyranose, chondroitin sulfate, chondrosamine, chondrosine, chrysophanic acid, colominic acid, convallatoxin, alpha-cyclodextrine, beta-cyclodextrine, cytidine 5'-diphosphoglucose, cytosine 1-beta-arabinofuranoside, daunosamine, n-decyl-beta-glucopyranoside, 5-deoxyarabinose, 2-deoxy-2-fluoroglucose, 3-deoxy-3-fluoroglucose, 4-deoxy-4-fluoroglucose, 6-deoxygalactopyranose, 2-deoxygalactose, 1-deoxyglucohex-1-eno-pyranose tetrabenzoate, 2-deoxyglucose, 6-deoxyglucose, 2-deoxyglucose 6-phosphate, 1-deoxymannojirimycin, 6-deoxymannose, 1-deoxy-1-morpholinofructose, 1-deoxy-1-nitroalutol, 1-deoxy-1-nitroaltitol, 1-deoxy-1-nitrogallactitol, 1-deoxy-1-nitromannitol, 1-deoxy-1-nitrosorbitol, 1-deoxy-1-nitrotalitol, deoxynojirimycin, 3-deoxy-erythro-pentose, 2-deoxy-6-phosphogluconic acid, 2-deoxyribose, 3-deoxyribose, 2-deoxy-alpha-ribose 1-

phosphate, 2-deoxyribose 5-phosphate, 5-deoxyxylofuranose, dextran, dextransulfate, dextrine, dextrose, diacetonefructose, diacetone mannitol, 3,4-di-o-acetyl-6-deoxyglucal, di-o-acetyl rhamnal, 2,3-diamino-2,3-dideoxy-alpha-glucose, 6,9-diamino-2-ethoxyacridine lactate, 1,3:4,6-di-o-benzylidene mannitol, 6,6'-dideoxy-6,6'-difluorotrehalose, digalactosyl diglyceride, digalacturonic acid, (+)digitoxose, 6,7-dihydrocoumarin-9-glucoside, dihydroxyacetone, dihydroxyacetone phosphate, dihydroxyfumaric acid, dihydroxymalic acid, dihydroxytartaric acid, dihydrozeatinriboside, 2,3-diphosphoglycerolic acid, dithioerythritol, dithiothreitol, n-dodecyl beta-glucopyranoside, n-dodecyl beta-maltoside, dulcitol, elemi-gum, endotoxin, epifucose, erythritol, erythro-pentulose, erythrose, erythrose 4-phosphate, erythrulose, esculin, 17-beta-estradiol-3-glucuronide 17-sulfate, estriole glucuronide, estron beta-glucuronide, ethodin, ethyl 4-o-beta-D-galactopyranosyl)-beta-D-glucopyranoside, ethyl 2-acetamido-4-o-(2-acetamido-2-deoxy-beta-glucopyranosyl)-6-o-(alpha-fucopyranosyl)-2-deoxy-beta-glucopyranoside, ethyl 2-acetamido-2-deoxy-4-o-(4-o-alpha-galactopyranosyl-beta-galactopyranosyl)-beta-glucopyranoside, ethyl cellulose ethylene glycol chitin, ethyl 4-o-(4-o-alpha-galactopyranosyl-beta-galactopyranosyl)-beta-glucopyranoside, ethyl 4-o-beta-galactopyranosyl-beta-glucopyranoside, ethyl pyruvate, ethyl beta-thiogluconide, etiocholan-3alpha-ol-17-on glucuronide, ficoll, 6-fluoro-6-deoxyglucose, franguloside, fraxin, fructosazine, beta-(-)fructose, fructose-1,6-diphosphate, fructose-2,6-diphosphate, fructose-1-phosphate, fructose-6-phosphate, fucoidan, fucose, alpha-(-)-fucose-1-phosphate, fucosylamine, 2'-fucosyllactose, 3-fucosyllactose, fumaric acid, galactal,

galactitol, galactopyranosylamine, 3-o-beta-galactopyranosyl-arabinose, 4-o-beta-galactopyranosyl-fructofuranose, 4-o-(4-o-beta-galactopyranosyl beta-galactopyranosyl)-glucopyranose, 4-o-alpha-galactopyranosyl-galactopyranose, 6-o-beta-galactopyranosylgalactose, 4-o-(beta-galactopyranosyl)-alpha-mannopyranose, alpha-galactopyranosyl 1-phosphate, galactopyranosyl-beta-thio-galactopyranoside, (+)galactosamine, alpha-galactosamine 1-phosphate, alpha-galactose 1-phosphate, galactose 6-phosphate, galactose 6-sulfate, 6-(alpha-galactosido)glucose, galacturonic acid, beta-gentiobiose, glucan, glucitol, glucoheptonic acid, glucoheptose, glucoheptulose, gluconate 6-phosphate, gluconic acid, 1-o-alpha-glucopyranosyl-beta-fructofuranoside, 6-o-alpha-glucopyranosylfructose, 1-o-alpha-glucopyranosyl-alpha-glucopyranoside, 4-o-beta-glucopyranosylglucopyranose, 4-o-(4-o-[6-o-alpha-glucopyranosyl-alpha-glucopyranosyl]-alpha-glucopyranosyl) glucopyranose, (+)glucosamine, alpha-glucosamine 6-2,3-disulfate, alpha-glucosamine 1-phosphate, glucosamine 6-phosphate, glucosamine 2-sulfate, alpha-glucosamine 3-sulfate, glucosamine 6-sulfate, glucosaminic acid, glucose, alpha-glucose 1,6-diphosphate, glucose 1-phosphate, glucose 6-phosphate, glucose 6-sulfate, glucuronamide, glucuronic acid, alpha-glucuronic acid 1-phosphate, glyceraldehyde, glyceraldehyde 3-phosphate, glycerate 2,3-diphosphate, glycerate 3-phosphate, glyceralic acid, alpha-glycerophosphate, beta-glycerophosphate, glycogen, glycolaldehyde, glycol chitosan, n-glycolylneuraminic acid, glycyric acid, glyoxylic acid, guanosine, 5'-diphosphoglucose, gulose, gums (accroides, agar, arab, carrageenan, damar, elemi, ghatti, guaiac, guar, karaya, locust bonne, mast, pontianac, storax, tragacanth, xanthan), heparin and heparin-like substances

(mesoglycan, sulodexide, etc.), heptakis (2,3,6-tri-o-methyl)-beta-cyclodextrin, heptanoyl-N-methylglucamide, n-heptyl beta-glucopyranoside, hesperidin, n-hexyl-beta-glucopyranoside, hyaluronic acid, 16-alpha-hydroxyestronglucuronide, 16-beta-hydroxyestron glucuronide, hydroxyethyl starch, hydroxypropylmethyl-cellulose, 8-hydroxyquinolin-beta-glucopyranoside, 8-hydroxyquinolin glucuronide, idose, (-)-idose, indole-3-lactic acid, indoxyl-beta-glucoside, epi-inositol, myo-inositol, myo-inositol bisphosphate, myo-inositol-1,2-cyl phosphate, scyllo-inositol, inositolhexaphosphate, inositolhexasulfate, myo-insoitol 2-monophosphate, myo-inositol trisphosphate, (q)-epi-inosose-2, scyllo-inosose, inulin, isomaltose, isomaltotriose, isosorbid dinitrate, 11-ketoandrosterone beta-glucuronide, 2-ketogluconic acid, 5-ketogluconic acid, alpha-ketopropionic acid, lactal, lactic acid, lactitol, lactobionic acid, lacto-N-tetraose, lactose, alpha-lactose 1-phosphate, lactulose, laminaribiose, laminnarine, levoglucosan, beta-levulose, lichenan, linamarine, lipopolysaccharides, lithiumlactate, lividomycine A, lyxose, lyxosylamine, maltitol, maltoheptaose, maltohexaose, maltooligosaccharide, maltopentaose, maltose, alpha-(+)maltose 1-phosphate, maltotetraose, maltotriose, malvidine-3,5-diglucoside, mandelonitril beta-glucoside, mandelonitril glucuronic acid, mannan, mannit, mannitol, mannitol 1-phosphate, alpha-mannoheptitol, mannoheptulose, 3-o-alpha-mannopyranosyl-mannopyranose, alpha(+)mannopyranosyl-1-phosphate, mannosamine, mannosan, mannose, A(+)mannose 1-phosphate, mannose 6-phosphate, (+)melezitose, A(+)melibiose, mentholglucuronic acid, 2-(3'-methoxyphenyl)-N-acetylneuraminic acid, methyl 3-o-(2-acetamido-2-deoxy-beta-galactopyranosyl)-alpha-

galactopyranoside, methyl 4-o-(3-o-[2-acetamido-2-deoxy-4-o-beta-galactopyranosyl beta-glucopyranosyl]-beta-galactopyranosyl)-beta-glucopyranoside, methyl 2-acetamido-2-deoxy-beta-glucopyranoside, methyl 3-o-(2-acetamido-2-deoxy-beta-glucopyranosyl)-beta-galactopyranoside, methyl 6-o-(2-acetamido)-2-deoxy-beta-glucopyranosyl)-alpha-mannopyranoside, methyl acosaminide, methyl alpha-altropyranoside, methyl 3-amino-3-deoxy-alpha-mannopyranoside, methyl beta-arabinopyranoside, methyl 4,6-o-benzylidene-2,3-di-o-toluenesulfonyl-alpha-galactopyranoside, methyl 4,6-o-benzylidene-2,3-di-o-p-toluenesulfonyl-alpha-glucopyranoside, methyl cellulose, methyl alpha-daunosaminide, methyl 6-deoxy-alpha-galactopyranoside, methyl 6-deoxy-beta-galactopyranoside, methyl 6-deoxy-alpha-glucopyranoside, methyl 6-deoxy-beta-glucopyranoside, methyl 3,6-di-o-(alpha-mannopyranosyl)-alpha-mannopyranoside, 1-o-methyl-alpha-galactopyranoside, 1-o-methyl-beta-galactopyranoside, methyl 3-o-alpha-galactopyranosyl-alpha-galactopyranoside, methyl 3-o-beta-galactopyranosyl-beta-galactopyranoside, 4-o-(2-o-methyl-beta-galactopyranosyl) glucopyranose, methyl 4-o-beta-galactopyranosyl-beta-glucopyranoside, methyl 4-o-(beta-galactopyranosyl-alpha-mannopyranoside, 5-5-methylgalactopyranose, methylgalactoside, n-methylglucamine, 3-o-methyl-alpha-glucopyranose, 1-o-methyl-alpha-glucopyranoside, 1-o-methyl-beta-glucopyranoside, alpha-methyl glucoside, beta-methyl glucoside, methyl glycol chitosan, methyl-alpha-mannopyranoside, methyl-2-o-alpha-mannopyranosyl-alpha-mannopyranoside, methyl 3-o-alpha-mannopyranosyl-alpha-mannopyranoside, methyl-4-o-alpha-mannopyranosyl-alpha-mannopyranoside, methyl 6-o-alpha-mannopyranosyl-alpha-mannopyranoside, methyl alpha-rhamnopyranoside, methyl alpha-ribofuranoside, methyl beta-ribofuranoside,

methylbeta-thiogalactoside, methyl 2,3,5-tri-o-benzoyl-
 alpha-arabinofuranoside, 4-methylumbelliferyl2-
 acetamido-4,6-o-benzylidene-2-deoxy-beta-glucopyranoside,
 4-methylumbelliferyl N-acetyl-beta-galactosaminide, 4-
 methylumbelliferyl N-acetyl-alpha-glucosaminide, 4-
 methylumbelliferyl-N-acetyl-beta-glucosaminide, 4-methy-
 lumbelliferyl-alpha-arabinofuranoside, 4-methylum-belli-
 feryl-alpha-arabinopyranoside, 4-methylum-belliferyl-
 beta-cellobioside, 4-methylumbelliferyl-beta-n,n'-diace-
 tylchitobioside, 4-methylumbelliferyl alpha-fucoside, 4-
 methylumbelliferyl beta-fucoside, 4-methylumbelliferyl
 alpha-galactopyranoside, 4-methylumbelliferyl beta-
 galactopyranoside, 4-methylumbelliferyl alpha-galacto-
 side, 4-methylumbelliferyl beta -glucopyranoside, 4-
 methylumbelliferyl alpha-glucoside, 4-methylumbelliferyl
 beta-glucoside, 4-methylumbelliferyl beta-glucuronide,
 4-methylumbelliferyl beta-mannopyranoside, 4-methylum-
 belliferylbeta-n,n',n''-triacylchitotriose, 4-methyl-
 umbelliferyl2,3,5-tri-o-benzyl-alpha-arabinofuranoside,
 4-methylumbelliferyl beta-xyloside, methyl beta-
 xylopyranoside, 2-o-methylxylose, alpha-methylxyloside,
 beta-methylxyloside, metrizamide, 2'-monophosphoadenosine
 5'-diphosphoribose, 2'-monophosphoinosine 5'-
 diphosphoribose, mucine, muraminic acid, naringine,
 sodium lactate, sodium polypectate, sodium pyruvate,
 neoagarobiose, neoagarohexaitol, neoagarohexaose,
 neoagarotetraose, beta-neocarrabiose, neocarrabiose
 4/1-sulfate, neocarrahexaose(2/4,4/1,4/3,4/5)-
 tetrasulfate, neocarratetraose(4/1,4/3)-disulfate,
 neocarratetraose(4/1)-sulfate, neohesperidin,
 dihydrochalcon, neohesperidose, neuraminic acid,
 neuraminic acid beta-methylglycoside, neuramine-lactose,
 nigeran, nigerantetrasaccharide, nigerose, n-nonyl
 glucoside, n-nonylbeta-glucopyranoside, octadecylthio-
 ethyl 4-o-alpha-galactopyranosyl-beta-galactopyranoside,

octadecylthioethyl 4-o-(4-o-[6-o-alpha-glucopyranosyl-
 alpha-glucopyranosyl]-alpha-glucopyranosyl)-beta-
 glucopyranoside, octanoyl n-methylglucamide, n-octyl
 alpha-glucopyranoside, n-octyl-beta-glucopyranoside,
 oxidised starch, pachyman, palatinose, panose,
 pentaerythritol, pentaerythritol diformal, 1,2,3,4,5-
 pentahydroxy, capronic acid, pentosanpolysulfate,
 perseitol, phenolphthalein glucuronic acid,
 phenolphthalein mono-beta-glucosiduron phenyl 2-
 acetamido-2-deoxy-alpha-galactopyranoside, phenyl 2-
 acetamido-2-deoxy-alpha-glucopyranoside, alpha-phenyl-
 N-acetyl-glucosaminide, beta-phenyl N-acetyl-
 glucosaminide, phenylethyl beta-galactoside, phenyl
 beta-galactopyranoside, phenyl beta-galactoside,
 phenyl alpha-glucopyranoside, phenyl beta-gluco-
 pyranoside, phenyl alpha-glucoside, phenyl beta-
 glucoside, phenyl beta-glucuronide, beta-phenyllactic
 acid, phenyl alpha-mannopyranoside, beta-phenylpyruvic
 acid, phenyl beta-thiogalactopyranoside, phenyl beta-
 thiogalactoside, phospho(enol)pyruvate, (+)2-
 phosphoglyceric acid, (-)3-phosphoglyceric acid,
 phosphohydroxypyruvic acid, 5-phosphorylribose 1-
 pyrophosphate, phytic acid, poly-N-acetylglucosamine,
 polygalacturonic acid, polygalacturonic acid methyl
 ester, polypectate, sodium, polysaccharide, 5beta-
 pregnane-3alpha,20alpha-diol glucuronide, n-propyl 4-o-
 beta-galactopyranosyl-beta-glucopyranoside, prunasine,
 psicose, pullulan, quinolyl-8beta-glucuronic acid,
 (+)raffinose, alpha-rhamnose, rhapontine, ribitol,
 ribonolacton, ribose, D-2-ribose, alpha-ribose 1-
 phosphate, ribose 2-phosphate, ribose 3-phosphate, ribose
 5-phosphate, ribulose, ribulose-1,5-diphosphate, ribulose
 6-phosphate, saccharic acid, saccharolactic acid,
 saccharose, salicin, sarcolactic acid, schardingers-
 alpha-dextrine, schardingers-beta-dextrine,

sedoheptulosan, sedoheptulose 1,7-diphosphate, sialic acid, sialyllactose, sinigrine, sorbitol, sorbitol 6-phosphate, (+)-sorbose, (-)sorbose, stachyose, starch, storax, styrax, sucrose, sucrose monocaprato, tagatose, alpha-talose, (-)-talose, tartaric acid, testosterone-beta-glucuronide, 2,3,4,6-tetra-o- methyl-glucopyranose, thiodiglucoside, 1-thio-beta- galactopyranose, beta-thiogluco-
 se, 5-thiogluco-
 se, 5- thiogluco-
 se 6-phosphate, threitol, threose, (+)threose, (-)threose, thymidine 5'-diphosphogluco-
 se, thymine 1-beta- arabinofuranoside, tragacanth, (+)trehalose, trifluorothymine, deoxyriboside, 3,3',5-trihydroxy-4'- methoxy-stilbene-3-o-beta-gluco-
 side, trimethylsilyl(+)-arabinose, trimethylsilyldulcitol, trimethylsilyl-beta (-) fructose, trimethylsilyl(+)-galactose, trimethylsilyl-alpha-(+)-glucose, trimethylsilyl(+)-mannitol, trimethylsilyl(+)-rhamnose, trimethylsilyl(-) sorbitol, trimethylsilyl(+)-xylose, rac-1-o-tritylglycerol, (+)turano-
 se, n-undecyl beta-gluco-
 pyranoside, uracil beta-arabinofuranoside, uridine 5'-diphospho-N-acetylglucosamine, uridine 5'-diphospho-
 galactose, uridine 5'-diphosphogluco-
 se, uridine 5'-diphospho-
 glucuronic acid, uridine 5'-diphosphomannose, uridine 5'-diphosphoxylose, vancomycin, xanthan gum, xylane, xylitol, xylitol, xylobiose, alpha-xylopyranosyl 1-phosphate, xylose, alpha-xylose 1-phosphate, xylose 5-phosphate, xylotriose, xylulose, xylulose 5-phosphate, yacca, zeatine riboside, zinclactate, zymosan A, etc.

Denotations desoxyribonucleic-(DNA) and ribonucleic acid (RNA) have their common meaning; preferably such DNA or RNA forms, or their antagonists, are used which have a particularly strong biological action.

- at least one nucleotide, peptide, protein or a related compound;

Nucleotides, which can be effectively transported with the aid of transfersomes, encompass adenine, adenosine, adenosine-3',5'-cyclic monophosphate, N6,02'-dibutyryl, adenosine-3',5'-cyclic monophosphate, N6,02'-dioctanoyl, adenosine, n6-cyclohexyl, salts of adenosine-5'-diphosphate, adenosine-5'-monophosphoric acid, adenosine-5'-o-(3-thiotriphosphate), salts of adenosine-5'-triphosphate, 9-beta-D-arabinoturanosyladenine, 1-beta-D-arabinoturanosylcytosine, 9-beta-D-arabinoturanosylguanine, 9-beta-D-arabinoturanosylguanine 5'-triphosphate, 1-beta-D-arabinoturanosylthymine, 5-azacytidine, 8-azaguanine, 3'-azido-3'-deoxythymidine, 6-benylaminopurine, cytidine phosphoramidite, beta-cyanoethyl diisopropyl, 249802cytidine-5'-triphosphate, 2'-deoxyadenosine, 2'-deoxyadenosine 5'-triphosphate, 2'-deoxycytidine, 2'-deoxycytidine 5'-triphosphate, 2'-deoxyguanosine, 2'-deoxyguanosine 5'-triphosphate, 2',3'-dideoxyadenosine, 2',3'-dideoxyadenosine 5'-triphosphate, 2',3'-dideoxycytidine, 2',3'-dideoxycytidine 5'-triphosphate, 2',3'-dideoxyguanosine, 2',3'-dideoxyguanosine 5'-triphosphate, 2',3'-dideoxyinosine, 2',3' dideoxythymidine, 2',3'-dideoxythymidine 5'-triphosphate, 2',3'-dideoxyuridine, N6-dimethylallyl-adenine, 5-fluoro-2'-deoxyuridine, 5-fluorouracil, 5-fluorouridin, 5-fluorouridine 5'-monophosphate, formycin A 5'-triphosphate, formycin B, guanosine-3'-5'-cyclic monophosphate, guanosine-5'-diphosphate-3'-diphosphate, guanosine-5'-o-(2-thiotriphosphate), guanosine-5'-o-(3'-thiotriphosphate), guanosine 5'-triphosphate, 5'-guanylylimidodiphosphate, inosine, 5-iodo-2'-deoxyuridine, nicotinamide-adenine dinucleotides, nicotinamide-adenine dinucleotides, nicotinamide-adenine dinucleotide phosphate, oligodeoxythymidylic acid, (p(dT)10), oligodeoxythymidylic acid (p(dT)12-18), polyadenylic acid

(poly A), polyadenylic acid-oligodeoxythymidynic acid, polycytidylic acid, poly(deoxyadenyl-deoxythymidylic acid, polydeoxyadenylic-acid-oligodeoxythymidynic acid, polydeoxythymidylic acid, polyinosine acid-polycytidylic acid, polyuridynic acid, ribonucleic acid, tetrahydro-uridine, thymidine, thymidine-3',5'-diphosphate, thymidine phosphoramidite, beta-cyanoethyl diisopropyl, 606102 thymidine 5'-triphosphate, thymine, thymine riboside, uracil, uridine, uridine-5'-diphosphoglucose, uridine 5'-triphosphate, xanthine, zeatine, transeatine riboside, etc. Further suitable polymers are: poly(DA) ss, poly(A) ss, poly(C) ss, poly(G) ss, poly(U) ss, poly(DA)-(DT) ds, complementary homopolymers, poly (D(A-T)) ds, copolymers, poly(DG)-(DC) ds, complementary homopolymers, poly (d(G-C)) ds copolymers, poly (d(L-C)) ds copolymers, poly(I)-poly(C) ds, etc. An oligopeptide or a polypeptide preferably contains 3-250, frequently 4-100, and very often 4-50 amino acids which are mutually coupled via amide-bonds. Suitable amino acids are usually of the alpha- and L-type; exceptions, however, such as in dermorphine are possible.

Peptides with a particularly high biological and/or therapeutic significance, and which can also be combined with transfersomes, are, for example, N-acetyl-Ala-Ala-Ala-, N-acetyl-Ala-Ala-Ala methyl ester, N-acetyl-Ala-Ala-Ala-Ala, N-acetyl-Asp-Glu, N-acetyl-Gly-Leu, N-alpha-Acetyl-Gly-Lys methyl ester acetate, acetyl-hirudine fragments, acetyl-5-hydroxy-Trp-5-hydroxy-Trp amide, des-acetyl-alpha-melanocyte stimulating hormone, N-Acetyl-Met-Asp-Arg-Val-Leu-Ser-Arg-Tyr, N-acetyl-Met-Leu-Phe, acetyl-muramyl-Ala-isoGln, N-acetyl-Phe-Tyr, N-acetyl-Phe-norLeu-Arg-Phe amide, N-acetyl-renine substrate tetradecapeptide, N-acetyl-transforming growth factor, adipokinetic hormone II, adjuvant peptide,

adrenal peptide E, adrenocorticotrophic hormone (ACTH 1-39, Corticotropine A) and its fragments such as 1-4 (Ser-Tyr-Ser-Met), 1-10 (Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly), 1-17, 1-24 and 1-39, 11-24, 18-39, Ala-Ala, beta-Ala-Ala, Ala-Ala-Ala, Ala-Ala-Ala methyl ester, Ala-Ala-Ala-Ala, Ala-Ala-Ala-Ala-Ala, Ala-Ala-Ala-Ala-Ala-Ala, Ala-Ala-Phe, 7-amido-4-methylcoumarin, Ala-Ala-Phe p-nitroanilide, Ala-Ala-Val-Ala p-nitroanilide, Ala-Arg-Pro-Gly-Tyr-Leu-Ala-Phe-Pro-Arg-Met amide, beta-Ala-Arg-Ser-Ala-Pro-Thr-Pro-Met-Ser-Pro-Tyr, Ala-Asn, Ala-Asp, Ala-Glu, Ala-gamma-Gln-Lys-Ala-Ala, Ala-Gly, beta-Ala-Gly, Ala-Gly-Glu-Gly-Leu-Ser-Ser-Pro-Phe-Tyr-Ser-Leu-Ala-Ala-Pro-Gln-Arg-Phe amide, Ala-Gly-Gly, Ala-Gly-Ser-Glu, Ala-His, beta-Ala-His, Ala-isoGln-Lys-Ala-Ala, Ala-Ile, Ala-Leu, beta-Ala-Leu, Ala-Leu-Ala, Ala-Leu-Ala-Leu, Ala-Leu-Gly, Ala-Lys, beta-Ala-Lys, Ala-Met, N-beta-Ala-1-methyl-His, Ala-norVal, Ala-Phe, beta-Ala-Phe, Ala-Phe-Lys 7-amido-4-methylcoumarin, Ala-Pro, Ala-Pro-Gly, Ala-sarcosine, Ala-Ser, Ala-Ser-Thr-Thr-Thr-Asn-Tyr-Thr, Ala-Ser-Thr-Thr-Thr-Asn-Tyr-Thr amide, Ala-Thr, Ala-Trp, beta-Ala-Trp, Ala-Tyr, Ala-Val, beta-Ala-Val, beta-Ala-Trp-Met-Asp-Phe amide, alytesine, amanitine, amastatine, angiotensine I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu), II II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe), III and related peptides, angiotensine II antagonist, angiotensine II receptor binding protein, angiotensine converting enzyme and its inhibitor (e.g. entipaine, bestatine, chymostatine, E-64, elastatinal, etc.) anserine, antide, aprotinine, arginine, vasopressine-Ala-Gly, Arg-Ala, Arg-Arg-Leu-Ile-Glu-Asp-Ala-Glu-Tyr-Ala-Ala-Arg-Gly, Arg-Asp, Arg-Glu, Arg-Gly, Arg-Gly-Asp, Arg-Gly-Asp-Ser, Arg-Gly-Asp-Ser-Pro-Ala-Ser-Ser-Lys-Pro, Arg-Gly-Glu-Ser, Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Ala, Arg-His-Phe, Arg-Ile, Arg-Leu, Arg-Lys, Arg-Lys-Asp-Val-Tyr, Arg-Phe, Arg-Phe-Asp-Ser, Arg-Pro-Pro-Gly-Phe-Ser-

Pro-Phe-Arg, Arg-Ser-Arg, Arg-Ser-Arg-His-Phe, Arg-Val, Asn-Pro-Asn-Ala-Asn-Pro-Asn-Ala, Asn-Pro-Asn-Ala-Asn-Pro-Asn-Ala-Asn-Pro-Asn-Ala, alpha-Asp-Ala, Asp-Ala-Glu-Asn-Leu-Ile-Asp-Ser-Phe-Gln-Glu-Ile-Val, Asp-Asp, alpha-Asp-Glu, alpha-Asp-Gly, beta-Asp-Gly, beta-Asp-His, Asp-Leu amide, beta-Asp-Leu, alpha-Asp-Lys, alpha-Asp-Phe amide, alpha-Asp-Phe, alpha-Asp-Phe methyl ester, beta-Asp-Phe methyl ester, alpha-Asp-Ser-Asp-Pro-Arg, Asp-Val, beta-Asp-Val, atrial natriuretic peptide, especially its fragments 1-32 and 5-28, atriopeptine I, II and III, auriculine A and B, beauvericine, beniotript, bestatine, N-benzylated peptides, big gastrin I, bombesin, (D-Phe¹²,Leu¹⁴) (Tyr⁴), (Lys³)-bombesin, (Tyr⁴)-bombesin, adrenal medulla docosa peptide and dodeca peptide, Bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) and related peptides, Bradykinin potentiators, brain natriuretic peptide, buccaline, bursine, S-t-butyl-Cys, caeruleine, calcitonine, calcitonine gene related peptide I and II, calmoduline binding domain, N-carboxymethyl-Phe-Leu, N-((R,S)-2-carboxy-3-phenyl-propionyl)Leu, cardioactive peptides A and B, carnosine, beta-casomorphine, CD4, cerebelline, N-chloroacetyl-Gly-Gly, chemotactic peptides such as formylated substances, cholecystokinin fragments, e.g., cholecystokinin octapeptide, coherine etc.

Also worth mentioning are the collagen peptides, conicostatine, conicotropine releasing factor, conotoxin G1, M1, and GVIA, corticotropine-like intermediate lobe peptide, corticotropine releasing factor and related peptides, C-peptide, Tyr-C-peptide, cyclic calcitonine gene related peptides, cyclo(His-Phe-), cyclo(His-Pro-), cyclo(Leu-Gly-), cyclo(Pro-Gly-), Cys-Asp-Pro-Gly-Tyr-Ile-Ser-Arg amide, Cys-Gln-Asp-Ser-Glu-Thr-Arg-Thr-Phe-Tyr, DAGO, Delta-sleep inducing peptide, dermorphine,

(Ser(Ac)7)-dermorphine, diabetes associated peptide and its amide, N-alpha,N-epsilon-diacetyl-Lys-Ala-Ala, N-2,4-dinitrophenyl-Pro-Gln-Gly-Ile-Ile-Gly-Gln-Arg, diprotine A, dynorphines such as dynorphine A (Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-sn-Gln), fragments 1-6 (leucine enkephaline-Arg), 1-8, 1-13 or E-64, dynorphine B, ebelactones (e.g. A and B) ecarine, elastatinal, elendoisine and related peptides, alpha-, beta- und gamma-endorphine, endothelins, endorphines (e.g. alpha (=beta-Lipotropine 61-76), (Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr), beta (=beta-Lipotropine 61-91) and other beta-lipotropine-fragments, enkephaline and Leu-enkephaline (Tyr-Gly-Gly-Phe-Leu) and related peptides, enkephalinase inhibitors (e.g. epimastatine, epibestatine, foroxymithine, leupeptine, pepstatine, Nle-Sta-Ala-Sta), eosinophilotactic tetrapeptide, epimastatine, epibestatine, (Cys(Acm)20,31)-epidermal growth factor and its fragments or receptors, epidermal mitosis inhibiting pentapeptide, trans-epoxysuccinyl-Leu amido-(4-guanidino)butane, erythropoietine and fragment, S-ethylglutathione, fibrinogen related peptide, fibrinopeptide A and B, Tyr-fibrinopeptide A, (Glu1)-fibrinopeptide S, fibrinopeptide B-Tyr, fibroblast growth factor fragment 1-11, follicular gonadotropine releasing peptide, N-formylated peptides, foroxymithine, N-(3(2-furyl)acryloyl) peptide derivatives, galanine, GAP 1-13, gastric inhibitory polypeptide, gastrine related peptides and derivatives, gastrine releasing peptide, gastrointestinal peptides (e.g. Ala-Trp-Met-Asp-Phe-Amid, bombesine, caeruleine, cholecystokinin, gelanine, gastrine, glucagon, motiline, neuropeptide K, pancreatic polypeptide, pancreozymin, Phi-27, secretine, valosine, etc.), Gln-Ala-Thr-Val-Gly-Asp-Val-Asn-Thr-Asp-Arg-Pro-Gly-Leu-Leu-Asp-Leu-Lys, (des-His1, Glu9)-glucagon amide, glucagon (1-37),

glucagon-like peptide I, alpha-Glu-Ala, Glu-Ala-Glu, Glu-Ala-Glu-Asn, alpha-Glu-Glu, gamma-Glu-Glu, gamma-Glu-Gln, gamma-Glu-Gly, PGlu-Gly-Arg-Phe amide, alpha-Glu-Gly-Phe, gamma-Glu-His, gamma-Glu-Leu, alphaGlu-alpha-Lys, gamma-Glu-epsilon-Lys, N-gamma-Glu-Phe, PGlu-Ser-Leu-Arg-Trp amide, alpha-Glu-Trp, gamma-Glu-Trp, gamma-Glu-Tyr, alpha-Glu-Val, gamma-Glu-Val, PGlu-Val-Asn-Phe-Ser-Pro-Gly-Trp-Gly-Thr amide, A-Glu-Val-Phe, glutathiones and related peptides, glutathionesulfonic acid, Gly-Ala, Gly-beta-Ala, Gly-Ala-Ala, Gly-Ala-Ala-Ala-Ala, Gly-Ala-Tyr, Gly-alpha-aminobutyric acid, Gly-gamma-aminobutyric acid, Gly-Arg-Ala-Asp-Ser-Pro-Lys, Gly-Arg-Ala-Asp-Ser-Pro-OH, Gly-Arg-Gly-Asp-Ser, Gly-Arg-Gly-Asp-Asn-Pro-OH, Gly-Arg-Gly-Asp-Ser-OH, Gly-Arg-Gly-Asp-Ser-Pro-Lys, Gly-Arg-Gly-Asp-Ser-Pro-OH, Gly-Arg-Gly-Asp-Thr-Pro, Gly-Arg-Gly-Asp-Thr-Pro-OH, Gly-Arg p-nitroanilide, Gly-Arg-Gly-Asp, Gly-Arg-Gly-Asp-Ser, Gly-Asn, Gly-Asp, Gly-Asp-Asp-Asp-Asp-Lys, Gly-Glu, Gly-Gly and their derivatives such as methyl, ethyl or benzyl esters or amides, Gly-Gly-Ala, Gly-Gly-Arg, Gly-Gly-Gly, Gly-Gly-Gly-Gly, Gly-Gly-Gly-Gly-Gly, Gly-Gly-Gly-Gly-Gly-Gly, Gly-Gly-Ile, Gly-Gly-Leu, Gly-Gly-Phe, Gly-Gly-Phe-Leu, Gly-Gly-Phe-Leu amide, Gly-Gly-Phe-Met, Gly-Gly-Phe-Met amide, Gly-Gly-sarcosine, Gly-Gly-Tyr-Arg, Gly-Gly-Val, Gly-His, Gly-His-Arg-Pro, Gly-His-Gly, Gly-His-Lys, Gly-His-Lys-OH, Gly-Ile, Gly-Leu amide, Gly-Leu, Gly-Leu-Ala, Gly-Leu-Phe, Gly-Leu-Tyr, Gly-Lys, Gly-Met, Gly-norLeu, Gly-norVal, Gly-Phe amide, Gly-Phe, Gly-Phe-Ala, Gly-Phe-Arg, Gly-Phe-Leu, Gly-Phe-Phe, Gly-Pro, Gly-Pro-Ala, Gly-Pro-Arg, Gly-Pro-Arg-Pro, Gly-Pro-Arg-Pro-OH, Gly-Pro-Gly-Gly, Gly-Pro-hydroxy-Pro, Gly-sarcosine, Gly-Ser, Gly-Ser-Phe, Gly-Thr, Gly-Trp, Gly-Tyr amide, Gly-Tyr, Gly-Tyr-Ala, Gly-Val, Gly-Phe-Ser, granuliberine R, growth hormone releasing factor and its fragments, Hexa-Ala, Hexa-Gly, Hippuryl-Arg (Hip-Arg), Hippuryl-Gly-Gly (Hip-

Gly-Gly), Hippuryl-His-Leu (Hip-His-Leu), Hippuryl-Lys, Hippuryl-Phe, hirudine and its fragments, His-Ala, His-Gly, His-Leu, His-Leu-Gly-Leu-Ala-Arg, His-Lys, His-Phe, His-Ser, His-Tyr, HIV envelope protein (gp120), Hydra peptides, P-hydroxyhippuryl-His-Leu, hypercalcemia malignancy factor (1-40), insulin chains B and C, P-iodo-Phe, Ile-Asn, Ile-Pro-Ile, insulin-like growth factor I (especially fragment 1-70), insulin-like growth factor II (especially its fragment 33-40), interleukin-1B fragment 163-171, isotocine, kassinine (Asp-Val-Pro-Lys-Ser-Asp-AGly-n-Phe-Val-Gly-Leu-Met-NH₂) katacalcine (calcitonine precursor peptide), Tyr-katacalcine, kemptide, kentsine, kyotorphine, laminine nonapeptide, laminine pentapeptide, laminine pentapeptide amide, leucine encephaline and related peptides, leucopyrokinine, Leu-Ala, Leu-beta-Ala, Leu-Arg, Leu-Asn, leucokinin I (Asp-Pro-Ala-Phe-Asn-Ser-Trp-Gly-NH₂) and II, Leucine-encephaline amide (Leu-encephaline amide) and related peptides, Leu-Gly, Leu-Gly-Gly, Leu-Gly-Phe, Leu-Leu amide, Leu-Leu, Leu-Leu-Leu amide, Leu-Leu-Leu, Leu-Leu-Phe amide, Leu-Leu-Tyr, Leu-Lys-Lys-Phe-Asn-Ala-Arg-Arg-Lys-Leu-Lys-Gly-Ala-Ile-Leu-Thr-Thr-Met-Leu-Ala, Leu-Met, Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys, Leu-Phe, Leu-Pro, Leu-Pro-Pro-Ser-Arg, Leu-Ser, Leu-Ser-Phe, Leu-Trp, Leu-Tyr, Leu-Val, leucotriene, Leu-Leu methyl ester, leupeptin, Leu-Ser-p-nitro-Phe-Nle-Ala-Leu methyl ester, beta-lipotropin fragments, litorine, luteinizing hormone releasing hormone and related peptides, lymphocyte activating pentapeptide, Lys-Ala, Lys-Ala 7-amido-4-methylcoumarin, Lys-Asp, Lys-Cys-Thr-Cys-Cys-Ala, Lys-Glu-Glu-Ala-Glu, Lys-Gly, Lys-Leu, Lys-Lys, Lys-Met, Lys-Phe, Lys-Pro-Pro-Thr-Pro-Pro-Pro-Glu-Pro-Glu-Thr, Lys-Serum thymic factor, Lys-Trp-Lys, Lys-Tyr-Trp-Trp-Phe amide, Lys-Val, macrophage inhibitory peptide (Tuftsine

fragment 1-3, Thr-Lys-Pro), magainine I and II, mast cell degranulating peptide, mastoparane, alpha1-mating factor, Melanine-Concentrating Hormone, MCD peptide, alpha-, beta-, gamma-, and delta-melanocyte stimulating hormones and related peptides, melittine, mesotocine, Met-beta-Ala, Met-Asn-Tyr-Leu-Ala-Phe-Pro-Arg-Met amide, methionine enkephaline and related peptides, Met-Ala, Met-Ala-Ser, Met-Asn, methionine-enkephaline (Met-enkephaline, Tyr-Gly-Gly-Phe-Met) and related peptides, methionine-enkephaline amide (Met-Enkephaline amide, Tyr-Gly-Gly-Phe-Met-NH₂) and related peptides, Met-Gln-Trp-Asn-Ser-Thr-Thr-Phe-His-Gln-Thr-Leu-Gln-Asp-Pro-Arg-Val-Arg-Gly-Leu-Tyr-Phe-Pro-Ala-Gly-Gly, Met-Glu, Met-Gly, Met-Leu, Met-Leu-Phe, Met-Lys, Met-Met, Metorphamide, Met-Phe, Met-Pro, Met-Ser, Met-Tyr-Phe amide, Met-Val, N-Methoxycarbonyl-Nle-Gly-Arg, P-nitroaniline, methoxysuccinyl-Ala-Ala-Pro-Val, methoxysuccinyl-Ala-Ala-Pro-Val 7-amido-4-methylcoumarin, Met-somatotropine, molluscan cardioexcitatory peptide, morphiceptine, (Val3)-morphiceptine, motiline, MSH-release inhibiting factor, myeline basic protein or its fragments, naphthylamide-derivatives of various peptides, beta-naphthyl-Ala-Cys-Tyr-Trp-Lys-Val-Cys-Thr amide, alpha-neoendorphine, beta-neoendorphine, alpha-neurokinin, neurokinin A, (substance K, neuromedin L) and B, neoendorphine (alpha: Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro, beta, etc.) neuromedin B, C, K, U8, U-25 etc., neurokinin A and B, neuropeptides K and Y, neurophysin I and II, neurotensine and related peptides, nitroanilide peptide derivatives, Nle-Sta-Ala-Sta, NorLeu-Arg-Phe amide, opioid peptides (e.g. adrenal peptide E, Ala-Gly-Glu-Gly-Leu-Ser-Ser-Pro-Phe-Trp-Ser-Leu-Ala-Ala-Pro-Gln-Arg-Phe-amides, casein fragments, casomorphine, N-CBZ-Pro-D-Leu, dermorphine, kyotorphine, morphiceptine (Tyr-Pro-Phe-Pro-NH₂), meorphamide (Tyr-Gly-Gly-Phe-Met-Arg-Arg-Val, adrenorphine),

osteocalcin (esp. its fragment 7-19), oxytocine and related peptides, pancreastatine and its fragments, such as 33-49, pancreatic polypeptide, pancreozymin, parathyroid hormone or fragments thereof, especially 1-34 and 1-84, penta-Ala, penta-Gly, penta-Phe, pepstatin A, peptide YY, peptide T, phalloidin, Phe-Ala-Ala-p-nitro-Phe-Phe-Val-Leu 4-pyridylmethyl ester, Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe amide, Phe-Ala, Phe-Gly, Phe-Gly-Gly, Phe-Gly-Gly-Phe, Phe-Gly-Phe-Gly, Phe-Leu amide, Phe-Leu, Phe-Leu-Arg-Phe amide, Phe-Leu-Glu-Glu-Ile, Phe-Leu-Glu-Glu-Leu, Phe-Leu-Glu-Glu-Val, Phe-Met, Phe-Met-Arg-Phe amide, Phe-Phe, Phe-Phe-Phe, Phe-Phe-Phe-Phe, Phe-Phe-Phe-Phe-Phe, Phe-Pro, Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Arg, Phe-Tyr, Phe-Val, PHI-27, PHM-27, phosphoramidone, physalaemine (pGlu-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-Gly-Leu-Met-NH₂), preproencephaline fragment 128-140, pressinoic acid and related peptides, Pro-Asn, proctoline (Arg-Tyr-Leu-Pro-Thr), proencephaline, Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-Lys, Pro-Ala, Pro-Arg 4-methoxy-beta-naphthylamide, Pro-Asp, proglumide, Pro-Gly, Pro-Gly-Gly, Pro-hydroxy-Pro, Pro-Ile, Pro-Leu, Pro-Leu-Gly amide, Pro-Met, Pro-Phe amide, Pro-Phe, Pro-Phe-Arg 7-amido-4-methylcoumarin, Pro-Phe-Gly-Lys, Pro-Trp, Pro-Tyr, Pro-Val, cyclic AMP dependent protein kinase and its inhibitors, PyroGlu-Ala-Glu, PyroGlu-Ala, PyroGlu-Ala-Glu, PyroGlu-Asn-Gly, PyroGlu-Gly-Arg p-nitroanilide, PyroGlu-His-Gly amide, PyroGlu-His-Gly, PyroGlu-His-Pro amide, PyroGlu-His-Pro, PyroGlu-Lys-Trp-Ala-Pro, ranatensine, renine substrate tetradecapeptide, N-(alpha-rhamnopyranosyloxy-hydroxyphosphinyl) Leu-Trp, sarcosyl-Pro-Arg p-nitroanilide, sauvagine, sleep-inducing peptide (Trp-Ala-Gly-Gly-Asp-Ala-Ser-Gly-Glu), secretine and related peptides, Ser-Ile-Gly-Ser-Leu-Ala-Lys, Ser-Ser-Ser, serum thymic factor, Ser-Ala, Ser-beta-Ala, Ser-Asn, Ser-Asp, Ser-Asp-Gly-Arg-Gly, Ser-Glu, Ser-Gln, Ser-Gly,

Ser-His, Ser-Leu, Ser-Met, Ser-Phe, Ser-Ser-Ser, Ser-Tyr, sleep inducing peptide, somastotine and related peptides (e.g. cyclo(p-Trp-Lys-Trh-Phe-Pro-Phe), steroido-genesis activator polypeptide, substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH₂) and related peptides, N-succinyl-derivatives of various peptides, syndyphalin-20 (Tyr-D-Met(O)-Gly-Phe-ol), tentoxin, tetra-Ala, tetra-Gly, thiostrepton, DL-thiorphane (encephalinase inhibitor), Thr-beta-Ala, Thr-Asp, Thr-Leu, Thr-Lys-Pro-Arg, Thr-Ser, Thr-Ser-Lys, Thr-Tyr-Ser, Thr-Val-Leu, thymopoietin fragments, thymosin alpha1 and its fragments, thymus circulating factor, thyrocalicitonin, thyrotropin releasing hormone, tocinoic acid, tosylated peptides, transforming growth factors, Tri-Ala, Tri-Ala methyl ester, Trp-Ala, Trp-Ala-Trp-Phe amide, Trp-Glu, Trp-Gly, Trp-Gly-Gly, Trp-His-Trp-Leu-Gln-Leu, Trp-His-Trp-Leu-Gln-Leu-Lys-Pro-Gly-Gln-Pro-Met-Tyr, Trp-His-Trp-Leu-Ser-Phe-Ser-Lys-Gly-Glu-Pro-Met-Tyr, Trp-Leu, Trp-Met-Asp-Phe amide, Trp-norLeu-Arg-Phe amide, Trp-Phe, Trp-Trp, Trp-Tyr, Tuftsin (Thr-Lys-Pro-Arg) and its fragments, Tyr-Ala, Tyr-Ala-Gly, Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu, Tyr-Ala-Gly-N-methyl-Phe 2-hydroxyethylamide, Tyr-Ala-Phe-Met amide, Tyr-Arg, Tyr-atriopeptin II, Tyr-Glu, Tyr-Gly, Tyr-Gly-Ala-Val-Val-Asn-Asp-Leu, Tyr-Gly-Gly, Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Arg, Tyr-Gly-Gly-Phe-Met-Arg-Arg-Val amide, Tyr-Gly-Trp-Phe-Phe amide, Tyr-Leu, Tyr-Phe, Tyr-Phe-Met-Arg-Phe amide, Tyr-Phe-Phe amide, Tyr-Pro-Leu-Gly amide, Tyr-Pro-Phe-Pro amide, Tyr-Pro-Val-Pro amide, Tyr-Thr-Gly-Leu-Phe-Thr, Tyr-Tyr-Phe amide, Tyr-Trp-Ala-Trp-Phe amide, Tyr-Trp-Ala-Trp-Phe methylamide, Tyr-Tyr-Leu, Tyr-Tyr-Phe, Tyr-Tyr-Tyr, Tyr-Tyr-Tyr methyl ester, Tyr-Tyr-Tyr-Tyr-Tyr-Tyr, Tyr-Val amide, Tyr-Val, Tyr-Val-Gly, Urodilatin, Urotensin II, Valosin, Val-Ala, Val-Ala p-nitroanilide, Val-Ala-Ala-Phe, Val-Asp, Val-Glu, Val-Gln, Val-Glu-Glu-Ala-Glu, Val-Glu-Ser-Ser-Lys,

Val-Gly, Val-Gly-Asp-Gln, Val-Gly-Gly, Val-Gly-Ser-Glu, Val-Gly-Val-Ala-Pro-Gly, Val-His-Leu-Thr-Pro, Val-His-Leu-Thr-Pro-Val-Glu-Lys, Val-Leu, Val-Lys, Val-Met, Val-Phe, Val-Pro, Val-Pro-Asp-Pro-Arg, Val-Pro-Leu, Val-Ser, Val-Thr, Val-Trp, Val-Tyr, Val-Tyr-Val, Val-Val, vasoactive intestinal peptides and related peptides, vasopressin related peptides, vasotocin and related peptides, xenopsin, etc.

Extended polypeptides are normally called proteins, independent of their detailed conformation. In this description, this term denotes, by and large, an enzyme or a coenzyme, an adhesion- or a recognition molecule, such as a CAMP or an OMP or a lectin, a histocompatibility complex, such as MHC-I or MHC-II, or an immunoglobuline (antibody) - or any (bio)chemical or (molecular)genetic modification thereof. Particularly useful for the applications according to this invention are the (bio)chemical modifications in which individual proteins are substituted with apolar residues, such as an alkyl, acyl, alkenoyl, etc. chains; but this is not a stringent limitation.

An enzyme is a catalytically active protein. Enzymes are normally grouped according to their basic functions. The most important enzymes for this invention are (E.C. numbers are given in brackets):

Oxidoreductases, such as: alcohol dehydrogenase (1.1.1.1), alcohol dehydrogenase (NADP dependent) (1.1.1.2), glycerol dehydrogenase (1.1.1.6), glycerophosphate dehydrogenase (1.1.1.8), xylulose reductase (1.1.1.10), polyol dehydrogenase (1.1.1.14), sorbitol dehydrogenase (1.1.1.14), myo-inositol dehydrogenase (1.1.1.18), uridine 5'-diphosphoglucose dehydrogenase

(1.1.1.22), glyoxalate reductase (1.1.1.26), lactate dehydrogenase (1.1.1.27), lactate dehydrogenase (1.1.1.28), glycerate dehydrogenase (1.1.1.29), beta-hydroxybutyrate dehydrogenase (1.1.1.30), beta-hydroxyacyl CoA dehydrogenase (1.1.1.35), malate dehydrogenase (1.1.1.37), malate enzyme (1.1.1.40), isocitric dehydrogenase (1.1.1.42), 6-phosphogluconate dehydrogenase (1.1.1.44), glucose dehydrogenase (1.1.1.47), beta-galactose dehydrogenase (1.1.1.48), glucose-6-phosphate dehydrogenase (1.1.1.49), 3alpha-hydroxysteroid dehydrogenase (1.1.1.50), 3beta-hydroxysteroid dehydrogenase (1.1.1.51), 3alpha,2beta-hydroxysteroid dehydrogenase (1.1.1.53), 3-phosphoglycerate dehydrogenase (1.1.1.95), fucose dehydrogenase (1.1.1.122), lactate dehydrogenase (cytochrome) (1.1.2.3), glucose oxidase (1.1.3.4), cholesterol oxidase (1.1.3.6), galactose oxidase (1.1.3.9), alcohol oxidase (1.1.3.13), glycolate oxidase (1.1.3.15), choline oxidase (1.1.3.17), glycerol-3-phosphate oxidase (1.1.3.21), xanthine oxidase (1.1.3.22), alcohol dehydrogenase (1.1.99.8), fructose dehydrogenase (1.1.99.11), formaldehyde dehydrogenase (1.2.1.1), formate dehydrogenase (1.2.1.2), aldehyde dehydrogenase (1.2.1.5), glyceraldehyde-3-phosphate dehydrogenase (1.2.1.12), gabase (1.2.1.16), pyruvate oxidase (1.2.3.3), oxalate oxidase (1.2.3.4), dihydroorotate dehydrogenase (1.3.3.1), lipoxidase (1.3.11.12), alanine dehydrogenase (1.4.1.1), glutamic dehydrogenase (1.4.1.3), glutamate dehydrogenase (NADP) (1.4.1.4), L-amino acid oxidase (1.4.3.2), D-amino acid oxidase (1.4.3.3), monoaminoxidase (1.4.3.4), diaminoxidase (1.4.3.6), dihydrofolate reductase (1.5.1.3), 5,10-methylenetetrahydrofolat dehydrogenase (1.5.1.5), saccharopine dehydrogenase NAD⁺ (1.5.1.7), octopine dehydrogenase (1.5.1.11), sarcosine oxidase (1.5.3.1),

sarcosine dehydrogenase (1.5.99.1), glutathione reductase (1.6.4.2), ferridoxin-NADP⁺ reductase (1.6.7.1), NADPH-FMN oxidoreductase (1.6.99.1), cytochrome c reductase (1.6.99.3), NADH-fmn oxidoreductase (1.6.99.3), dihydropteridin reductase (1.6.99.7), uricase (1.7.3.3), diaphorase (1.8.1.4), lipoamide dehydrogenase (1.8.1.4), cytochrome oxidase (1.9.3.1), nitrate reductase (1.9.6.1), phenolase (1.10.3.1), ceruloplasmine (1.10.3.2), ascorbate oxidase (1.10.3.3), NADH peroxidase (1.11.1.1), catalase (1.11.1.6), lactoperoxidase (1.11.1.7), myeloperoxidase (1.11.1.7), peroxidase (1.11.1.7), glutathione peroxidase (1.11.1.9), chloroperoxidase (1.11.1.10), lipoxidase (1.13.1.12), protocatechuate 3,4-dioxygenase (1.13.11.3), luciferase (glow-worm) (1.13.12.7), salicylate hydroxylase (1.14.13.7), p-hydroxybenzoate hydroxylase (1.14.13.2), luciferase (bacterial) (1.14.14.3), phenylalanine hydroxylase (1.14.16.1), dopamine-beta-hydroxylase (1.14.17.1), tyrosinase (1.14.18.1), superoxide dismutase (1.15.1.1), ferredoxine-NADP reductase (1.18.1.2), etc.. Transferases, such as: catecholic o-methyltransferase (2.1.1.6), phenylethanolamine N-methyl-transferase (2.1.1.28), aspartate transcarbamylase (2.1.3.2), ornithine carbamyltransferase (2.1.3.3), transketolase (2.2.1.1), transaldolase (2.2.1.2), choline acetyltransferase (2.3.1.6), carnitine acetyltransferase (2.3.1.7), phosphotransacetylase (2.3.1.8), chloroamphenicol acetyltransferase (2.3.1.28), kanamycine 6'-acetyltransferase (2.3.1.55), gentamicine acetyltransferase (2.3.1.60), transglutaminase (2.3.2.13), gamma-glutamyl transpeptidase (2.3.2.2), phosphorylase A (2.4.1.1), phosphorylase B (2.4.1.1), dextran sucrose (2.4.1.5), sucrose phosphatase (2.4.1.7), glycogen synthase (2.4.1.11), uridine 6'-diphosphoglucuronyltransferase (2.4.1.17), galactosyl trans-

ferase (2.4.1.22), nucleoside phosphorylase (2.4.2.1), orotidine-5'-monophosphate pyrophosphorylase (2.4.2.10), glutathione S-transferase (2.5.1.18), glutamine-oxalate transaminase (2.6.1.1), glutamic-pyruvate transaminase (2.6.1.2), gabase (2.6.1.19), hexokinase (2.7.1.1), galactokinase (2.7.1.6), fructose-9-phosphate kinase (2.7.1.11), gluconate kinase (2.7.1.12), phosphoribulokinase (2.7.1.19), NAD kinase (nicotinamide adenine dinucleotide kinase) (2.7.1.23), glycerokinase (2.7.1.30), choline kinase (2.7.1.32), protein kinase (3':5'-cyclic-AMP dependent) (2.7.1.37), phosphorylase kinase (2.7.1.38), pyruvate kinase (2.7.1.40), fructose-9-phosphate kinase (pyrophosphate dependent) (2.7.1.50), acetate kinase (2.7.2.1), carbamate kinase (2.7.2.2), 3-phosphoglyceric phosphokinase (2.7.2.3), creatine phosphokinase (2.7.3.2), etc.

Transpeptidases, such as: esterase (3.1.1.1), lipase (3.1.1.3), phospholipase A (3.1.1.4), acetyl esterase (3.1.1.6), cholinesterase, acetyl (3.1.1.7), choline-esterase, butyryl (3.1.1.8), pectinesterase (3.1.1.11), cholesterol esterase (3.1.1.13), glyoxalase ii (3.1.2.6), phosphatase, alkaline (3.1.3.1), phosphatase acid (3.1.3.2), 5'-nucleotidase (3.1.3.5), 3'-nucleotidase (3.1.3.6), glucose-6-phosphatase (3.1.3.9), fructose-1,6-diphosphatase (3.1.3.11), phytase (3.1.3.26), phosphodiesterase i (3.1.4.1), glycerophosphorylcholine (3.1.4.2), phospholipase C (3.1.4.3), phospholipase D (3.1.4.4), deoxyribonuclease I (3.1.4.5), deoxyribonuclease II (3.1.4.6), ribonuclease N1 (3.1.4.8), sphingomyelinase (3.1.4.12), phosphodiesterase 3':5'-cyclic (3.1.4.17), phosphodiesterase II (3.1.4.18), endonuclease (3.1.4.21), ribonuclease A (3.1.4.22), ribonuclease B (3.1.4.22), 3'-phosphodiesterase 2':3'-cyclic nucleotide (3.1.4.37), sulfatase (3.1.6.1), chondro-4-sulfatase (3.1.6.9),

chondro-6-sulfatase (3.1.6.10), ribonuclease T2
 (3.1.27.1), ribonuclease T1 (3.1.27.3), ribonuclease u2
 (3.1.27.4), nuclease (3.1.30.1), nuclease, (from
 micrococces) (3.1.31.1), alpha-amylase (3.2.1.1), beta-
 amylase (3.2.1.2), amyloglucosidase (3.2.1.3), cellulase
 (3.2.1.4), laminarinase (3.2.1.6), dextranase (3.2.1.11),
 chitinase (3.2.1.14), pectinase (3.2.1.15), lysozyme
 (3.2.1.17), neuraminidase (3.2.1.18), alpha-glucosidase,
 maltase (3.2.1.20), beta-glucosidase (3.2.1.21), alpha-
 galactosidase (3.2.1.22), beta-galactosidase (3.2.1.23),
 alpha-mannosidase (3.2.1.24), beta-mannosidase
 (3.2.1.25), invertase (3.2.1.26), trehalase (3.2.1.28),
 beta-N-acetylglucosaminidase (3.2.1.30), beta-glucuroni-
 dase (3.2.1.31), hyaluronidase (3.2.1.35), beta-
 xylosidase (3.2.1.37), hesperidinase (3.2.1.40),
 pullulanase (3.2.1.41), alpha-fucosidase (3.2.1.51),
 mycodextranase (3.2.1.61), agarase (3.2.1.81), endoglyco-
 sidase F (3.2.1.96), endo-alpha-N-acetylgalactosamini-
 dase (3.2.1.97), NADase (nicotinamide adenine glycopepti-
 dase) F (3.2.2.5), dinucleotidase (3.2.2.18), thiogluc
 (3.2.3.1), s-adenosylhomocystein-hydrolase (3.3.1.1),
 leucin-aminopeptidase, (from cytosol) (3.4.11.1), leucin-
 aminopeptidase, microsomale (3.4.11.2), pyroglutamate-
 aminopeptidase (3.4.11.8), carboxypeptidase a (3.4.12.2),
 carboxypeptidase B (3.4.12.3), prolidase (3.4.13.9),
 cathepsin C (3.4.14.1), carboxypeptidase W (3.4.16.1),
 carboxypeptidase A (3.4.17.1), carboxypeptidase B
 (3.4.17.2), alpha-chymotrypsin (3.4.21.1), beta-
 chymotrypsin (3.4.21.1), gamma-chymotrypsin (3.4.21.1),
 delta-chymotrypsin (3.4.21.1), trypsin (3.4.21.4),
 thrombin (3.4.21.5), plasmin (3.4.21.7), kallikrein
 (3.4.21.8), enterokinase (3.4.21.9), elastase from
 pancreas (3.4.21.11), protease (subtilisin) (3.4.21.14),
 urokinase (3.4.21.31), elastase from leucocytes
 (3.4.21.37), cathepsin B, (3.4.22.1), papain (3.4.22.2),

ficin (3.4.22.3), bromo-elain (3.4.22.4), chymopapain (3.4.22.6), clostripain (3.4.22.8), proteinase A (3.4.22.9), pepsine (3.4.23.1), renine (3.4.23.4), cathepsin D (3.4.23.5), protease (aspergillopeptidase) (3.4.23.6), collagenase (3.4.24.3), collagenase (3.4.24.8), pinguinain (3.4.99.18), renine (3.4.99.19), urokinase (3.4.99.26), asparaginase (3.5.1.1), glutaminase (3.5.1.2), urease (3.5.1.5), acylase i (3.5.1.14), cholyglycine hydrolase (3.5.1.24), urease(ATP-hydrolyzing) (3.5.1.45), penicillinase (3.5.2.6), cephalosporinase (3.5.2.8), creatininase (3.5.2.10), arginase (3.5.3.1), creatinase (3.5.3.3), guanase (3.5.4.3), adenosine-deaminase (3.5.4.4), 5'-adenylate acid-deaminase (3.5.4.6), creatinine deiminase (3.5.4.21), anorganic pyrophosphatase (3.6.1.1), adenosine 5'-triphosphatase (3.6.1.3), apyrase (3.6.1.5), pyrophosphatase, nucleotide (3.6.1.9), etc.

Lyases, such as: pyruvate-decarboxylase (4.1.1.1), oxalate decarboxylase (4.1.1.2), oxalacetate decarboxylase (4.1.1.3), glutamic decarboxylase (4.1.1.15), ornithine decarboxylase (4.1.1.17), lysine decarboxylase (4.1.1.18), arginin decarboxylase (4.1.1.19), histidine decarboxylase (4.1.1.22), orotidine 5'-monophosphate decarboxylase (4.1.1.23), tyrosine decarboxylase (4.1.1.25), phospho(enol) pyruvate carboxylase (4.1.1.31), ribulose-1,5-diphosphate carboxylase (4.1.1.39), phenylalanine decarboxylase (4.1.1.53), hydroxymandelonitrilelyase (4.1.2.11), aldolase (4.1.2.13), N-acetylneuramine acid aldolase (4.1.3.3), etc. citrate lyase (4.1.3.6), citrate synthase (4.1.3.7), tryptophanase (4.1.99.1), isozymes of carbonic anhydrase (4.2.1.1), fumarase (4.2.1.2), aconitase (4.2.1.3), enolase (4.2.1.11), crotonase (4.2.1.17), delta-amino-levulinate dehydratase (4.2.1.24), chondroitinase ABC

(4.2.2.4), chondroitinase AC (4.2.2.5), pectolyase (4.2.2.10), aspartase (4.3.1.1), histidase (4.3.1.3), phenylalanine ammonia-lyase (4.3.1.5), argininosuccinate lyase (4.3.2.1), adenylosuccinate lyase (4.3.2.2), glyoxalase II (4.4.1.5), isomerases, such as: ribulose-5'-phosphate 3-epimerase (5.1.3.1), uridine 5'-diphosphogalactose 4-epimerase (5.1.3.2), mutarotase (5.1.3.3), triosephosphate isomerase (5.3.1.1), phosphoriboisomerase (5.3.1.6), phosphomannose isomerase (5.3.1.8), phosphoglucose isomerase (5.3.1.9), tautomerase (5.3.2.1), phosphoglucomutase (5.4.2.2), ligases, e.g.: aminoacyl-tRNA synthetase (6.1.1), s-acetyl coenzyme A synthetase (6.2.1.1), succinic thiokinase (6.2.1.4), glutamine synthetase (6.3.1.2), pyruvate carboxylase (6.4.1.1), etc.

The following are, amongst others, referred to as proteases: aminopeptidase M, amino acid-arylamidase, bromo-elaine, carboxypeptidase A, carboxypeptidase B, carboxypeptidase P, carboxypeptidase Y, cathepsine C, chymotrypsine, collagenases, collagenase/dispase, dispase, elastase, endoproteinase Arg-c, endoproteinase Asp-n sequencing grade, encloproteinase Glu-c (proteinase V8), endoproteinase Glu-c sequencing grade, endoproteinase Lys-c, endoproteinase Lys-c sequencing grade, endoproteinas, factor Xa, ficine, kallikrein, leucine-aminopeptidase, papaine, pepsine, plasmin, pronase, proteinase K, proteinase V8 (endoproteinase Glu-c), pyroglutamate-aminopeptidase, pyroglutamate-aminopeptidase, restriction protease factor Xa, subtilisine, thermolysine, thrombine, trypsin, etc.

A coenzyme according to this invention is any substance which supports enzyme activity. Amongst the biologically important coenzymes are, for example, acetyl-coenzyme A,

acetylpyridine-adenine-dinucleotide, coenzyme A, flavine-adenine-dinucleotide, flavine-mononucleotide, NAD, NADH, NADP, NADPh, nicotinamide-mononucleotide, s-palmitoyl-coenzyme A, pyridoxal-5'-phosphoric acid, etc.

Another class of proteins, which are important in the context of this invention, are lectins. Plants, and sometimes also animal, tissues are suitable sources of lectins; particularly convenient sources are *Abrus precatorius*, *Agaricus bisporus*, *Agrostemma githago*, *Anguilla anguilla*, *Arachis hypogaea*, *Artocarpus integrifolia*, *Bandeiraea simplicifolia* BS-I und BS-II, (*Griffonia simplicifolia*), *Banhlula purpurea*, *Caragana arborescens*, *Cicer arietinum*, *Canavalia ensiformis* (jack bean), *Caragana arborescens* (Siberian pea tree), *Codium fragile* (green algae), *Concanavalin A* (Con A), *Cytisus scoparius*, *Datura stramonium*, *Dolichos biflorus*, *Erythrina corallodendron*, *Euonymus europaeus*, *Gelonium multiflorum*, *Glycine max* (soy), *Griffonia simplicifolia*, *Helix aspersa* (garden snail), *Helix pomatia* (escargot), *Laburnum alpinum*, *Lathyrus odoratus*, *Lens culinaris* (lentil), *Limulus polyphemus*, *Lycopersicon esculentum* (tomato), *Lotus tetragonolobus*, *Luffa aegyptiaca*, *Maclura pomifera* (Osage orange), *Momordica charantia* (bitter pear melon), *Naja mocambique* (Mozambiquan cobra), *Naja Naja kaouthia*, *Mycoplasma gallisepticum*, *Perseu americana* (avocado), *Phaseolus coccineus* (beans), *Phaseolus limensis*, *Phaseolus lunatus*, *Phaseolus vulgaris*, *Phytolacca americana*, *Pseudomonas aeruginosa* PA-I, *Pisum sativum* (pea), *Ptilota plumosa* (red algae), *Psophocarpus tetragonolobus* (winged bean), *Ricinus communis* (castor bean), *Robinia pseudoacacia* (false acacia, black locust), *Sambucus nigra* (clematis), *Saponaria officinalis*, *Solanum tuberosum* (potato), *Sophora japonica*, *Tetragonolobus purpureas* (winged or asparagus pea), (*Lotus tetragono-*

lobus), *Triticum vulgare* (wheat germ), *Ulex europaeus*, *Vicia faba*, *Vicia sativa*, *Vicia villosa*, *Vigna radiata*, *Viscum album* (mistle), *Wisteria floribunda*, etc.

Further interesting proteins are, e.g. the activator of tissue-plasminogen, insulin, kallikrein, keratin, kininogen, lactoterrin, laminarin, laminin, alpha2-macroglobulin, alpha1-microglobulin, F2-microglobulin, high density lipoproteins, basic myelin-protein, myoglobins, neurofilaments I, II, and III, neurotensin, oxytocin, pancreatic oncofetal antigen, parvalbumin, plasminogen, platelet factor 4, pokeweed antiviral protein, porphobilinogen, prealbumin, prostate specific antigens, protamine sulfate, protein C, protein C activator, protein S, prothrombin, retinol binding protein, S-100 protein, pregnancy protein-1, serum amyloid A, serum amyloid P component, tenascin, testosterone-estradiol binding globulin, thioredoxin, thrombin, thrombocytin, beta-thromboglobulin, thromboplastin, microsomal antigen from thyroid, thyroid stimulating hormone, thyroxine binding globulin, transcortin, transferrin, ubiquitin, vimentin, vinculin, vitronectin, etc.

Some typical examples of human and animal hormones which can be used as agents according to the invention are, for example, acetylcholine, adrenaline, adrenocorticotrophic hormone, angiotensin, antidiuretic hormone, cholecystikinin, chorionic gonadotropin, corticotropin A, danazol, diethylstilbestrol, diethylstilbestrol glucuronide, 13,14-dihydro-15-keto-prostaglandins, 1-(3',4'-dihydroxyphenyl)-2-aminoethanol, 5,6-dihydroxytryptamine, epinephrine, follicle stimulating hormone, gastrin, gonadotropin, 8-hypophamine, insulin, juvenile hormone, 6-ketoprostaglandins, 15-ketoprostaglandins,

LTH, luteinizing hormone releasing hormone, luteotropic hormone, α -melanocyte stimulating hormone, gamma-melanocyte stimulating hormone, 5-melanocyte stimulating hormone, noradrenaline, norepinephrine, oxytocine, parathyroid hormone, parathyroid substances, prolactine, prostaglandins, secretine, somatostatine, somatotropine (STH), thymosine alpha 1, thyrocalcitonine, thyroglobuline, thyroid stimulating hormone, thyrotropic hormone, thyrotropine releasing hormone, 3,3',5'-triiodothyroacetic acid, 3,3',5'-triiodothyronine, TSH, vasopressine, etc.

Oestrogens are mostly steroid hormones with 18 carbon atoms and one unsaturated (aromatic) ring. Amongst the most important oestrogens are, for example, chlorotrianisene, diencestrole, diethylstilboestrole, diethylstilboestrol-dipropionate, diethylstilboestrol-disulfate, dimestrole, estradiole, estradiolbenzoate, estradiolundecylate, estriolsuccinate, estrone, ethinglestradiole, nexoestrole, nestranole, oestradiolvalerate, oestriole and quinestrole.

Gestagenes are typically synthetic hormones, mainly with progesterone-like characteristics; the most important agents belonging to this class are allylestrenole, chloromadinonacetate, dimethisterone, ethisterone, hydroxyprogesteron-caproate, lynestrenole, medrogestone, medroxyprogesteron-acetate, megestrolacetate, methyloestrenolone, norethisterone, norethisterone-acetate, and norgestrel.

Agents can also be parts of a biological extract. As sources of biologically and/or pharmacologically active extracts, the following are worth-mentioning: for example, *Acetobacter pasteurianum*, *Acokanthera ouabaio*

cathel, Aesculus hippocastanum, Ammi visnaga Lam., Ampi Huasca, Apocynum Cannabium, Arthrobotrys superba var. oligospora (ATCC 11572), Atropa belladonna, Bacillus Lentus, Bacillus polymyxa, Bacillus sphaericus, Castilleja elastica cerv., Chondrodendron tomentosum (Ampi Huasca), Convallaria majalis, Coronilla-enzymes, Corynebacterium hoagii (ATCC 7005), Corynebacterium simplex, Curvularia lunata (Wakker) Boadijn, Cyllindrocarpon radicola (ATCC 11011), Cynara scolymus, Datura Metel, didymella, digilanidase, digitalis Lanata, digitalis purpurea, Duboisia, Flavobacterium dehydrogenans, Fusarium exquiseti saccardo, Hyoscyamus niger, Jaborandi-leaves (P. microphyllus Stapf), Micromonosporapurpurea u. echinospora, Paecilomyces varioti Bainier var. antibioticus, Penicillium chrysogenum Thom, Penicillium notatum Westling, Penicillium patulum, Rauwolfia serpentina Benth., Rhizopus arrhizus Fischer (ATCC-11145), Saccharomyces cerevisiae, Schizomycetes ATCC-7063, Scilla maritima L., Scillarenase, Septomyxa affinis (ATCC 6737), Silybum marianum Gaertn., Streptomyces ambofaciens, Strophantusgratus, Strophantus Kombe, Thevetia peruviana, Vinca minor L., Vinca rosea, etc.

Unless stated otherwise, all substances, surfactants, lipids, agents or additives with one or several chiral carbon atoms can be used either as a racemic mixture or in the form of optically pure enantiomers.

WORKING PRINCIPLE

The transport of agents through permeation barriers can be mediated by such carriers which fulfill the following basic criteria:

- carriers should experience or create a gradient which drives them into or through a barrier, e.g. from the body surface into or through the skin, or from the surface of a leaf into the depth of a leaf, or from one side of a barrier to the other;
- the resistance to permeation which is felt by the carriers in the barrier should be as small as possible in comparison to the driving force;
- carriers should be capable of permeating in and/or through a barrier without thereby losing their associated agents in an uncontrollable manner.

Carriers, moreover, should preferably provide control of the distribution of agents, as well as over the effectiveness and temporal development of the agents action. They should be capable of bringing materials into the depth of and across a barrier, if so desired, and/or should be capable of catalyzing such a transport. Last but not least, such carriers should affect the range and depth of action as well as the type of cells, tissue parts, organs and or system parts which can be reached or treated, under suitable conditions at least.

In the first respect, chemical gradients are especially convenient for biological applications. Particularly suitable are the physico-chemical gradients, such as the pressure of (de)hydration pressure (humidity gradient) or a difference in concentration between the sites of application and action; however, electrical or magnetic fields as well as thermal gradients are also interesting in this respect. In technological applications, an externally applied pressure or existing hydrostatic pressure difference are also of importance.

In order to fulfill the second condition, carriers must be sufficiently 'fluid' at the microscopic scale; this enables them to easily cross the constrictions in the permeability barrier.

Permeation resistance is a decreasing function of the decreasing carrier size. But also the carrier driving force frequently depends on the size of the permeating particle, droplet or vesicle; when the driving pressure is size-independent, the corresponding force also typically decreases with decreasing carrier size. This causes the transfer effectiveness to be a complex function of the carrier size, often showing a maximum depending on the chosen carrier and/or agent composition.

In the case of molecular aggregates the permeation resistance is largely determined by the mechanical elasticity and deformability of the carrier, the viscosity of the total preparation being also important, however. The former must be sufficiently high, the latter low enough.

Size and, even better, deformability can serve as a criterion for the optimization of the supramolecular carriers according to this invention. As an indication of deformability, the capacity of individual carriers to form protrusions can be studied, as a function of all relevant system parameters. (In practical terms, it is often sufficient to investigate only such variables which come into question for a controllable application. The examples given in this application, therefore, only pertain to varying the concentrations of the edge active components and the absolute carrier concentration which affect the forced diminishment of the lipid vesicle or of vesicle permeation.) This is true e.g. for transcutaneous and transcuticular transport as well as for the transport of agents through the lung alveoli, into the hair, into gels, and

the like.

With regard to the third requirement, the choice of the carriers, agents and additives, as well as the applied carrier dose or concentration all play some role. Low dose, in the majority of cases, gives rise to a predominantly surface treatment: poorly water-soluble substances in such case remain confined largely to the apolar region of a permeability barrier (such as in the epidermal membranes); agents which are highly soluble and can diffuse easily from the carriers can attain a distribution which is different from that of the carrier particles; for such substances, the permeability of a transfersomal membrane is also important. Edge active substances with a tendency to leave carriers and move into a barrier give rise to a locally variable carrier composition, etc. These interdependencies should be thought of and considered prior to each individual application. In the search for a set of conditions under which a simple carrier vesicle becomes a transfersome, the following rules of thumb can be used:

- At first, the conditions are determined under which the carrier vesicles are solubilized by the edge active substances. At this critical point the 'vesicles' are maximally deformable owing to the fact that they are permanently formed and deformed. At the same time, however, they are also unstable and incapable of holding and transferring water soluble substances.
- Next, the carrier composition or concentration is adapted by reducing the edge activity in the system to an extent which ensures the vesicle stability as well vesicle deformability to be sufficiently high; this also ensures the permeation capacity of such carriers to be satisfactory. The term stability in this application implies,

on the one hand, a mechanical tendency of the carrier components to "stay together"; on the other hand, that the carrier composition during the transport, and in particular during the permeation process, does not change at all or not much. The position of the corresponding optimum which one is looking for hereby depends on many boundary conditions. The type of agent molecules also plays an important role in this. The smaller and the more hydrophilic the agent to be transported, the further the carrier system must be spaced from the solubilization point; the desired shelf life of carriers is also important: upon approaching the solubilization point, the tendency of transfersomes to form larger particles may increase and the carrier's storage capacity simultaneously decrease.

- Ultimately, the system parameters need to be optimized with respect to the envisaged modes and goals of a given application. Rapid action requires a high permeation capability; in order to achieve slow drug release, it is advantageous to ensure gradual penetration through the permeability-barrier and a correspondingly 'finely adjusted' membrane permeability; in order to reach deep regions, high doses are needed; in order to obtain a broad distribution, it is recommended to use carrier concentrations which are not too high.

This application describes some relevant properties of the transfersomes as carriers for the lipid vesicles. Most of the examples pertain to carriers made of phospholipids, but the general validity of conclusions is not restricted to this carrier or molecule class. The vesicle examples should only illustrate the requirements which should be fulfilled in order to attain penetration through permeability barriers, such as skin. Similar properties, moreover, ensure carrier transport

across animal or human epidermis, mucosa, plant cuticle, inorganic membranes, etc.

The fact that the cells in a horny skin layer continuously merge with the watery compartments of subcutis is probably one reason for the spontaneous permeation of transfersomes through the 'pores' in this layer: during the permeation process transfersomes are propelled by the osmotic pressure. As an alternative, external pressures, such as an electroosmotic or hydrostatic pressure, however, can also be applied in addition.

Depending on the vesicle dose used, the dermally applied carrier particles can penetrate as deep as the subcutaneous layer. Agents can then be locally released, enriched in (the depth of) the application site, or forwarded to other tissues and body systems through a system of blood and lymph vessels, the precise drug fate being dependent on the carrier size, composition and formulation.

It is sometimes convenient to adjust the pH-value of a formulation immediately after it has been prepared or directly prior to an application. Such an adjustment should prevent the deterioration of individual system components and/or drug carriers under the conditions of initial pH; simultaneously, a physiological compatibility should be achieved. For the neutralization of carrier suspensions, physiologically tolerable acids or bases are most frequently used as well as buffers with a pH-value between 3-12, preferably 5 to 9 and most often 6-8, depending on the goal and site of application. Physiologically acceptable acids are, for example, diluted aqueous solutions of mineral acids, such as hydrochloric acid, sulfuric acid, or phosphoric acid, or organic acids, such as carboxyalkane acids, e.g. acetic acid. Physiologically acceptable bases are, for example, diluted sodium hydroxide,

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suitably ionized phosphoric acids, etc.

Formulation temperature is normally chosen to be well suited for the given substances; for aqueous preparations it is normally in the range of 0 to 95°C. Whenever possible, one should work in the temperature range 18-70°C; particularly preferred are temperatures between 15 and 55°C for the work with fluid chain lipids; the preferred temperature range for the lipids with ordered chains is from 45 to 60°C. Other temperature ranges are possible, however, most notably for the non-aqueous systems or preparations containing cryo- or heat-stabilizers.

If required by the sensitivity of one of the system components, transfersome formulations can be stored in cold (e.g. at 4°C). It is, moreover, possible to make and keep them under an inert atmosphere, e.g. under nitrogen. Shelf-life, furthermore, can be extended if no substances with multiple bonds are used, and if the formulation is (freeze) dried, or if a kit of dry starting materials is dissolved or suspended and processed at the site of application only.

In the majority of cases, carriers are applied at room temperature. But applications at lower or higher temperatures are also possible, especially when synthetic substances are used.

Transfersomal preparations can be processed previously or at the site of application, as has been described, for example, in our previous German patent application P 40 26 833.0-43, and exemplified in several cases in the handbook on 'Liposomes' (Gregoriadis, G., Edits. CRC Press, Boca Raton, Fl., Vols 1-3, 1987), in the monography 'Liposomes as drug carriers' (Gregoriadis, G., Edits. John Wiley & Sons, New York, 1988), or in the laboratory manual 'Liposomes. A Practical Approach' (New, R., Oxford-Press, 1989). If

required any suspension of drugs, moreover, can be diluted or concentrated (e.g. by per ultracentrifugation or ultrafiltration) immediately prior to a final application; additives can also be given into a preparation at this or a previous time. Upon any such manipulation, however, a possible shift of the permeation optimum for a given carrier preparation must be taken into account or prevented.

Transfersomes as described in this applications are well suited to be used as carriers of lipophilic substances, such as fat-soluble biological agents, therapeutics, poisons, etc. But it is quite likely that transfersomes used in combination with water soluble substances, especially when the molecular weight of the latter exceeds 1000 Dt, will be of even greater practical value.

Transfersomes, moreover, can contribute to the stabilization of substances which are sensitive to hydrolysis; they can improve carrier and drug distribution in the specimen and at the site of application and can also ensure a more favourable effect of the drug in time. Basic carrier ingredients can also bring advantages of their own. However, the most important carrier characteristics is the capability of transporting materials into and through a permeability barrier; this opens up a way for applications which prior to this discovery were not feasible.

The specific formulations as described in this invention have been optimized for the topical use on - or in the vicinity of - (a) permeability barrier(s). Particularly interesting barriers of this kind are skin and plant cuticle. (But formulations according to this invention are also well suited for the peroral (p.o) or parenteral (i.v. i.m. or i.p.) application, especially when edge active substances have been chosen in order to keep the drug loss at the site of

application low.) Edge active substances which have a diminished activity, are degraded preferentially, are absorbed particularly efficiently or are diluted strongly at the site of application are especially valuable in this last respect.

In dermatology, application doses of up to 50, often up to 10 and very frequently less than 2.5 (or even less than 1 mg) of carrier substance are used per cm^2 of skin surface, the given masses pertaining to the basic carrier substance. The optimal mass depends on the carrier composition, desired penetration depth and duration of action, as well as on the detailed application site. Application doses useful in agrotechnics are typically lower and frequently below 0.1g pro m^2 .

Depending on the goal of application, each formulation can also contain suitable solvents up to a total concentration which is determined by certain plausible physical (no solubilization or appreciable shift of penetration optimum), chemical (no lowering of stability), or biological and physiological (little adversary side effects) formulation requirements.

Quite suitable for this purpose are, for example, the unsubstituted or substituted, e.g. halogenated, aliphatic, cycloaliphatic, aromatic or aromatic-aliphatic hydrocarbons, such as benzol, toluol, methylene chloride or chloroform, alcohols, such as methanol or ethanol, propanediol, erithritol, short-chain alkane carboxylic acid esters, such as acetic acid alkylesters, such as diethylether, dioxan or tetrahydrofuran, or mixtures thereof.

A survey of the lipids and phospholipids which can be used for the applications as described in this report in addition to the ones already mentioned is given, for example, in 'Form and

function of phospholipids' (Ansell & Hawthorne & Dawson, eds.), 'An Introduction to the Chemistry and Biochemistry of Fatty Acids and Their Glycerides' of Gunstone and in other reference books. All implicitly and explicitly mentioned lipids and surfactants as well as other suitable edge active substances and their preparation are well known. A survey of available surfactants, together with the trademarks under which they are marketed by their manufacturers, is given in the annals 'Mc Cutcheon's, Emulsifiers & Detergents', Manufacturing Confectioner Publishing Co. An up-to-date compilation of the pharmaceutically acceptable agents is given, for example, in 'Deutsches Arzneibuch' (and in the annually updated list 'Rote Liste'); furthermore, in the British Pharmaceutical Codex, European Pharmacopoeia, Farmacopoeia Ufficiale della Repubblica Italiana, Japanese Pharmacopoeia, Nederlandse Pharmacopoeia, Pharmacopoeia Helvetica, Pharmacopée Française, The United States Pharmacopoeia, The United States NF, etc. A concise list of suitable enzymes can be found in the volume on 'Enzymes', 3rd Edition (M. Dixon and E.C. Webb, Academic Press, San Diego, 1979); more recent developments are described in the series 'Methods in Enzymology'. Many examples of the glycohydate-binding proteins which could be interesting for the use in combination with carriers as described in this invention are quoted in 'The Lectins: Properties, Functions, and Applications in Biology and Medicine' (I.E. Liener, N. Sharon, I.T. Goldstein, Eds. Academic Press, Orlando, 1986) as well as in the corresponding special publications; substances which are particularly interesting for agrotechnical applications are described, for example, in 'The Pesticide Manual' (C.R. Worthing, S.B. Walker, Eds. British Crop Protection Council, Worcestershire, Englande, 1986, e.g. 8th edition) and in 'Wirkstoffe in Pflanzenschutz und Schädlingsbekämpfung', which is published by Industrie-Verband Agrar (Frankfurt); most commonly available antibodies are listed in the catalogue

'Linscott's Directory', the most important neuropeptides in 'Brain Peptides' (D.T. Krieger, M.J. Brownstein, J.B. Martin, Eds. John Wiley, New York, 1983), corresponding supplementary volumes (e.g. 1987) and other special journals.

Methods for the preparation of liposomes, which in the majority of cases can also be used for manufacturing transfersomes, are described, for example, in 'Liposome Technology' (Gregoriadis, Ed., CRC Press) or older books dealing with similar topics, such as 'Liposomes in Immunobiology' (Tom & Six, Eds., Elsevier), 'Liposomes in Biological Systems' (Gregoriadis & Allison, Eds., Willey), 'Targeting of Drugs' (Gregoriadis & Senior & Trouet, Plenum), etc. Corresponding patent publications also are a valuable source of relevant information.

The following examples are aimed at illustrating this invention without restricting it. All temperatures are in degrees Celsius, carrier sizes in nanometers, pressures in Pascal and other units in standard SI system.

Ratios and percentages are given in moles, unless otherwise stated.

Examples 1-13:

Composition:

250-372 mg	phosphatidylcholine from soy-bean (+95 % = PC)
187-34.9 mg	oleic acid (+99 %)
0.312-0.465 ml	ethanol, absolute
10 mM	Hepes

Preparation:

Increasing amounts of oleic acid were pipetted into different volumes of alcoholic PC-solutions containing 75 micromoles of lipid so as to create a concentration series with a lipid/surfactant ratio beginning with $L/S=0.5$ and increasing by 0.2 units in each step. Subsequently, each lipid sample was supplemented with 4.5 ml of sterile buffer solution and the mixtures were incubated at 4°C for one day. When the pH value had to be adjusted by addition of 1 M NaOH, the first incubation period was followed by another incubation for 24 hours. In order to obtain a final liposome suspension, each sample was thoroughly mixed and filtered through a polycarbonate filter (0.45 micrometer) into a glass vial which was then kept closed at 4°C .

Characterization:

Permeation resistance is assumed to be proportional to the relative pressure needed to perform a secondary filtration through a 0.2 micrometer filter. In this report this resistance is given in relative units of 1 to 10.

Vesicle size is measured by means of dynamic light scattering at 33 degrees C, using a Malvern Zeta-Sizer instrument. For the analysis of correlation curves, a special variant of the software package "Contin" is employed.

In this experimental series all vesicle sizes are relatively independent of the total concentration of edge active substances, in the range of 300 through 350 nm.

Permeation:

Permeation resistance first increases with decreasing relative concentration of fatty acid in the transfersomes. This trend is not monotonous, however. At a lipid/surfactant-ratio of

approx. 2, the liposome permeation capacity starts to increase; but it then decreases again until, for L/S above 3, the transfersomes have nearly lost their capability for passing through narrow constrictions. Vesicles with a lipid/surfactant molar ratio of 1/2 are nearly perfectly permeable, however. (A suspension with 8 % lipid in such case can be filtered nearly as easily as pure water.). At this concentration ratio, which corresponds roughly to 30 % of the solubilization dose of fatty acids in an alkaline suspension, liposomes thus appear to correspond to optimal transfersomes.

Specific data points (0) are shown in figure 1. Vesicles diameters were always measured after permeation experiments.

Examples 14-20:

Composition:

349-358 mg	phosphatidylcholine from soy-bean (+95 % = PC)
63.6-52.2 mg	oleic acid (+99 %)
10 mM	Hepes

Preparation:

4.5 ml of buffer in each case are pipetted to a corresponding amount of lipids and fatty acids to create a concentration series with L/S = 1.92 through 2.4 in the steps of 0.08 units each; the pH value is set to 7.2-7.3 by 1 M NaOH. Lipid suspension after an incubation for 6 days at 4°C is treated by ultrasonication until vesicles with an average diameter of 0.8 micrometers are formed.

Permeation and Characterization:

Permeation resistance is determined as described in examples

1-13. Its value, as a function of the concentration of edge active substance in the system resembles the results of measurements 1-13. The resulting vesicles are somewhat larger than in the previous set of experiments, however, having diameters in the order of 500 nm. This can be explained by the relatively slow material flow during filtration.

Corresponding measured points are shown as (+) in figure 1.

Examples 21-31:

Composition:

322.6-372 mg	phosphatidycholine from soy-bean (+95 %=PC)
96.8-34.9 mg	oleic acid (+99 %)
0.403-0.465 ml	ethanol, absolute
10 mM	Hepes
130 mM	NaCl, p.a.

Preparation:

Preparation procedure used essentially corresponds to the one of examples 14-20. The main difference is that the electrolyte concentration in the present case was isotonic with blood.

Permeation and Characterization:

The measured permeation resistance corresponds, within the limits of experimental error, to the results given in examples 1-13. Vesicle sizes are also similar in both cases. Immediately after the lipid vesicle have been formulated, their diameters are in the range of 320-340 nm. 8 days later, however, the vesicle size has increased to approx. 440 nm.

Corresponding experimental data is given in figure 2.

Examples 32-39:

Composition:

184.5-199.8 mg	phosphatidylcholine from soy-bean (+95%=PC)
20.5-22.2 mg	phosphatidylglycerol from egg PC (puriss., Na-salt, =PG)
44.9-26.1 µl	oleic acid (+99 %)
0.165-0.178 ml	ethanol, absolute
4.5 ml	Hepes, 10 mM

Preparation:

Anhydrous PG is mixed with an alcoholic solution of PC to give a clear solution with 90 % PC and 10 % PG. Oleic acid is added to this solution; the resulting lipid/surfactant ratios are between 1.6 and 2.8; an isomolar specimen is made in addition to this. All mixtures are suspended in 4.5 ml of a sterile buffer solution to yield a final lipid concentration of 4 % and then left for 3 days, after a pH-value adjustment with NaOH, in order to age.

Permeation and Carrier Characteristics:

For determining the permeation resistance, the same procedure as in examples 1-13 is used. All measured values are, as a rule, smaller than in the case of carriers which contained no charged species but had a similar L/S-ratio. Based on our experiments with a 4 % suspension of PC and oleic acid we conclude that the relatively low total lipid concentration plays only a minor role in this respect.

As in previous examples, a resistance minimum is observed for

the 4 % PC/PG mixtures; this minimum, however, is found with L/S-ratios which are by some 20 % higher than those measured with 8 % lipid suspensions. Vesicle diameters, however, hardly differ from those measured in examples 1-13.

Precise permeation data is shown in figure 3. All quoted diameters were measured immediately after individual permeation experiments. But even 40 days later, they are hardly bigger than at the beginning; figure 4 illustrates this.

Examples 40-49:

Composition:

301.3-335.4 mg	phosphatidylcholine from soy-bean (+95%=PC)
123.3-80.8 μ l	Tween 80 (puriss.)
0.38-0.42 ml	ethanol, absolute
4.5 ml	phosphate buffer, isotonic, sterile

Preparation:

Increasing volumes of Tween 80 are pipetted into appropriate volumes of an alcoholic PC solution. This gives rise to a concentration series with 12.5 through 25 mol-% surfactant (L/S = 4-8). In addition to this, samples with L/S=2 and 3 are also made. After the addition of buffer, lipid vesicles are formed spontaneously: prior to further use, these are made somewhat smaller, with the aid of a 0.8 micrometer filter.

Permeation and Carrier Characteristics:

Permeation resistance is determined in the previously described manner. The corresponding values (0) are shown in the left part of figure 5. As in the case of transfersomes

which contain oleic acid, a region of anomalously high permeation capability (at $L/S = 6$) can be seen relatively far away from the solubilization point. But it is not before below $L/S=4$ that a maximum permeability is observed. The transfersomal optimum thus is located in a range which differs by a factor of 1.5-2 from the solubilization point.

Precise permeation data is given in figure 5 (wide lines, left panel). The experimental data in right panel documents the vesicle diameters determined after permeability measurements.

Examples 50-61:

Composition:

314.2-335.4 mg	soy-bean phosphatidylcholine (+95 % = PC)
107.2-80.8 μ l	Tween 80 (puriss.)
4.5 ml	phosphate buffer, isotonic, sterile

Preparation:

First Tween 80 and subsequently phosphate buffer are added to appropriate quantities of PC. The resulting mixture is agitated at room temperature for 4 days. The further procedure is as described in examples 40-49.

Permeation and Carrier Characteristics:

Corresponding permeability data is given in figure 5 (thin lines). It confirms, by and large, the results of experiments nos. 40-49.

Examples 62-75:

Composition:

193-361 mg phosphatidylcholine from soy-bean (grade I, S100)
207.2-38.8 mg Na-cholate, puriss.
4.5 ml phosphate buffer (isotonic with a physiologic
 solution)
 ethanol, absolute

Preparation:

0.5 ml of a hot solution of S100 in ethanol (2/1, M/V) are mixed with sufficient amounts of bile acid salts which give rise to a concentration series with increasing lipid/surfactant ratio between 1/2 and 5/1. The final total lipid concentration is 8 % in all cases.

Vesicle permeation through constrictions and vesicle solubilization:

The permeation resistance of each sample is measured as in examples 1-13. The vesicle size is determined by means of light scattering. (Radii of particles smaller than 5 nm cannot be measured owing to the insufficient power of the laser source used.)

Corresponding measured data is shown in figure 6. It indicates that the permeation resistance of transfersomes with an L/S ratio below 3.5/1 is very small but that this resistance increases significantly at higher L/S values (left panel); the increase of the mean vesicle diameter above L/S = 2.75 (right panel) is probably a consequence of the decreased flow (and thus of a diminished hydrodynamic shear) caused by the greater permeability resistance in this concentration range.

Within only a few hours after preparation the size of vesicles

just above the solubilization limit (at L/S between 1.25/1 and 2.5/1) is significantly bigger than in the vicinity the 'transfersome optimum'. Such undesired consequences of surfactant activity (cf. Fromherz, P. in: 'Galstone disease, Pathophysiology and Therapeutic Approaches', pp. 27-33, Springer, Berlin, 1990) should always be taken into account. At L/S of approx. 1.25/1, solubilization sets in which leads to the formation of, in our case unmeasurably, small mixed micelles of a size of approximately 5 nm.

Examples 76-91:

Composition:

1.627-0.5442 g phosphatidylcholine from soy-bean (grade I, S100)
4.373-0.468 g Na-cholate, puriss.
60 ml phosphate buffer (physiological)

Preparation:

A 10 % suspension of S100 in phosphate buffer is ultrasonicated at room temperature until the mean vesicle size is approx. 350 nm.

This suspension is divided into three equal volume parts containing 10 %, 1 % and 0.2 % phospholipids. Starting with these preparations, aliquots containing 5 ml of suspension each are prepared. These are supplemented with increasing amounts of sodium cholate (partly in the form of a concentrated micelle suspension), yielding a concentration series with L/S ratios between 1/5 and 5/1. Prior to each permeation- and solubilization measurement, the starting suspension is aged for 1 week at 4°C.

Vesicle permeation through constrictions and vesicle

solubilization:

In order to determine the permeation resistance of these samples two different procedures are used.

In the first series, each suspension is diluted prior to an actual measurement to get a final lipid concentration of 0.2 %; subsequently it is pressed through a filter with a pore size of 0.1 micrometers. The sample resistance is identified with the inverse value of the volume which has passed through the filter pores during a period of 5 minutes.

In the second series, the permeation resistance is determined as in examples 1-13 and finally renormalized by dividing the values thus obtained with regard to the final lipid concentration.

The resulting data shows that both the solubilization limit and the position of a 'transfersome optimum' expressed in terms of preferred L/S ratios are dependent on the overall lipid concentration. In the case of a 10 % suspension the corresponding values are approx. 1/1 and 2.75/1, respectively; for the 0.2 % suspension they increase to 1/4 and 1/1, however.

Examples 92-98:

Composition:

16.3-5.4 mg phosphatidylcholine from soy-bean (Grade I,
S100)
41.5-5.5 mg Na-desoxycholate, puriss.
5 ml phosphate buffer (physiological)

Preparation:

A suspension of 1 % desoxycholate containing vesicles is prepared as described in examples 76-91.

Vesicle permeation through constrictions and vesicle solubilization:

The measurements of this experimental series show that vesicles containing desoxycholate are solubilized already at L/S ratios near 1/2, i.e. at an L/S ratio which is by a factor of 2-3 lower than in the case of S100/Na-cholate vesicles.

Examples 99-107:

Composition:

3 mM Suspension of phosphatidylcholine from soy-bean (grade I, S100) in phosphate buffer Na-cholate, puriss.

Preparation:

A 3 mM suspension of S100 in phosphate buffer is partly homogenized at room temperature. 3 ml of this suspension are supplemented each with increasing amounts of sodium cholate in order to create a series with increasing L/S ratios between 1/2 and 12/1. After three days of incubation, these aliquots are ultrasonicated at 55°C, using a 50 % duty-cycle; simultaneously, the optical density at 400 nm of each sample is recorded. An analysis of the resulting experimental data within the framework of a bimodal exponential model reveals two characteristic vesicularization rates (τ_1 and τ_2); these characterize the temporal dependence of the number of lamellae in each vesicle (τ_1) and the changes in the mean size of vesicles (τ_2).

Vesicle characterization and deformability.

The tau 1 and tau 2 values represented in figure 7 show that the mechanical properties of transfersomes, which are reflected in the value of parameter tau 2, exhibit a similar L/S dependence as the solubilization and permeation tendency (cf. fig. 6). For a 0.2 % suspension investigated in this series 1 cholate molecule per lipid is required for a rapid formation of vesicles (for the formation of largely unilamellar vesicles).

Examples 108-119:Composition:

121.2-418.3 mg	phosphatidylcholine from soy-bean (Grade I, PC)
378.8-81.7 mg	Triton X-100
4.5 ml	0.9 % NaCl solution in water

Preparation:

A 10 % PC-suspension in isotonic solution of sodium chloride is homogenized at 22°C until the mean size of lipid vesicles is approx. 400 nm. This suspension is then distributed in aliquots of approx. 4.8 ml. A sufficient volume of Triton X-100 is pipetted into each of these aliquots to give a concentration series with nominal PC/Triton ratios in the range of 0.25 through 4 in steps of 0.5. All resulting samples are occasionally mixed and incubated at 4°C for 14 days.

Vesicle solubilization

The optical density (OD (400 nm)) of a lipid-triton mixture after a 10-fold dilution provides insight into the vesicle

solubilization; this is represented in the right panel of figure 8. The solubilization limit is approx. 2 triton molecules per PC-molecule. Right below this limit, the optical density (OD (400 nm)) - and thus the vesicle diameters - attain the greatest values. At PC/triton ratios higher than 2,5/1, the change in the optical density of given suspensions is only minimal.

Vesicle permeation and characteristics:

In order to evaluate the permeation capability of the resulting lipid vesicles and transfersomes all suspensions were pressed through fine-pore filters (0.22 micrometer), as described in examples 1-13. The required pressure increases gradually with the decreasing total triton concentration in the suspension; for L/S ratios higher than 2/1 this significantly limits the permeation capability of carriers.

Corresponding results are summarized in the left half of figure 8.

Examples 120-128:

Composition:

403,5-463,1 mg	dipalmitoyl tartaric acid ester, Na-salt
96,5-36,9 mg	laurylsulfate, Na-salt (SDS)
4,5 ml	triethanolamine buffer, pH 7.5

Preparation:

In this test series a synthetic lipid, which is not found in biological systems, was chosen to be the basic transfersome constituent. For each experiment the required dry lipid mass was weighed into a glass vial and mixed with 4.5 ml of buffer.

The latter contained sufficient amounts of sodiumdodecylsulfate (SDS) to give various L/S ratios between 2/1 and 6/1. Well mixed suspensions were first kept at room temperature for 24 hours and subsequently mixed again thoroughly.

Permeation capacity and vesicle characteristics:

Liposomes were pressed through a 0.2 micrometer filter. Simultaneously, the permeation resistance was measured. Vesicles with an L/S ratio below 4/1 can pass the membrane pores very easily; in contrast to this, all vesicles with lower surfactant concentrations or vesicles without edge active components can pass through the porous constrictions only with difficulty (not before an excess pressure of 5 MPa has been created) or not at all (membranes burst).

Examples 129-136:

Composition:

101,6-227 mg	phosphatidylcholine from soy-bean
148,4-22,2 mg	octyl-glucopyranoside (β -octylglucoside),
puriss. 9,85 ml	phosphate buffer, pH 7,3
	ethanol, absolute

Preparation:

Phosphatidylcholine in ethanol (50 %) and octyl-glucopyranoside were mixed in different relative ratios in order to prepare a concentration series with increasing L/S values between 1/4 and 2/1 (and a final total lipid concentration of 2.5 %). Each lipid mixture in a glass vial was then supplemented with 4.5 ml of buffer. Subsequently, the resulting suspension was mixed in an agitator for 48 hours

at 25°C. The suspension turbidity was greater for the specimen containing lower amounts of octylglucoside. A fine sediment formed in standing samples. Each suspension was mixed thoroughly before the experiment.

Vesicle permeation and characteristics:

All suspensions can be filtered without any problem through filters with a pore diameter of 0.22 micrometer, using only minimal excess pressures of less than 0.1-0.2 MPa; the only two exceptions are the samples with the lowest surfactant concentration. These give rise to small permeation resistances which on the renormalized scale (cf. figures 1-5) corresponds to values of approx. 1 and 2.5, respectively. Figure 9 presents said data.

If the pore diameter is reduced to 0.05 micrometers only suspensions with L/S ratios below 2/1 can still be filtered.

Irrespective of the pore size used all preparations with L/S ratios below 2/1 are unstable; after only a few days, a phase separation is observed between a micelle rich and a vesicle rich phase.

Examples 137-138:

Composition:

43,3 mg, 50 mg	phosphatidylcholine from soy-bean
0.5 mg	phosphatidylethanolamine-N-fluorescein
6,7 mg, 0 mg	cholate, Na-salt, p.a.
5 ml	Hepes-buffer, pH 7,3

Preparation:

Phosphatidylcholine with the addition of 1 %-fluoresceinated lipids with or without desoxycholate is suspended in 5 ml buffer. The lipid/surfactant ratio is 3.5/1 or 1/0. Both 1 %-suspensions are then ultrasonicated in a glass vial for 1.5 or 15 minutes (25 W, 20°C), until the mean vesicle size is approx. 100 nm.

Spontaneous vesicle permeation:

Onto a Millipore-filter with 0.3 micrometer pore diameter, mounted into a Swinney-holder, the lower half of which has been wetted and filled with water, 50 microliters of a lipid suspension are pipetted through the upper opening. By a gentle swinging motion, a relatively homogeneous sample distribution on the filter surface is ensured. After 30 minutes, the holder is carefully opened and left to dry for 60 minutes. Subsequently the water from below the filter is collected and checked fluorimetrically (excitation 490 nm, emission 590 nm). (The determined light intensity is a measure of the permeation capacity.)

The transport of fluorescence markers mediated by surfactants containing transfersomes gives rise to a fluorescence signal of 89.5; in control experiment a value of 44.1 is established. This indicates that transfersomes are capable of transporting encapsulated substances across permeability barriers.

Examples 137-139:

Composition:

43,5, 45,3, 50 mg	phosphatidylcholine from soy-bean
0.5 mg	phosphatidylethanolamine-N-fluorescein
6,5, 4,7, 0 mg	desoxycholate, Na-salt, p.a.

25 ml

Hepes-buffer, pH 7,3

Preparation and results:

Lipid vesicles are made and tested as described in examples 137-138. Measurements show that the transfersomes which contain deoxycholate already show similarly good results at a characteristic L/S ratio of 5/1 as transfersomes which contain cholate at a ratio of L/S=3.5.

Examples 140-142:

Composition:

50 mg;	43,3 mg;	15,9 mg	phosphatidylcholine from soy-bean
0.5 mg			phosphatidylethanolamine-N-fluorescein
0 mg;	6,7 mg;	34,1 mg	cholate, Na-salt, p.a.
5 ml			Hepes-buffer, pH 7,3

Preparation:

Lipid vesicles consisting of phosphatidylcholine and a fluorescent additive were made as in examples 137-138. For this experiment, suspensions with a lipid/surfactant ratio of 1/0, 4/1 and 1/4 were used. The former two contained fluorescent lipid vesicles, the latter a micellar suspension.

Spontaneous penetration into plant leaves:

A fresh onion is carefully opened in order to gain access to individual leaves; these correspond to low-chlorophyll plant leaves. For each measurement, 25 microliters of a fluorescinated suspension are applied onto the concave (inner or upper) side of each onion leaf; as a result of this a

convex droplet with an area of approx. 0.25 square centimeters is formed. (Carriers which contain surfactants can be easily identified owing to their higher wetting capability.) After 90 minutes the (macroscopically) dry lipid film is eliminated with the aid of a water stream from a jet-bottle with a volume of 50 ml.

After this treatment, the 'leaf surface' attains a slightly reddish appearance in the case of surfactant containing transfersomes as well as mixed micelles. Leaves incubated with surfactant-free vesicles cannot be distinguished from the untreated leaves.

Fluorescence measurements using a red filter (excitation through a blue filter from above) show that leaves which were covered with transfersomes are intensively fluorescent throughout the treated area. In certain places extremely brilliant aggregates are detected; these probably correspond to the non-eliminated vesicle-clusters. The fluorescence of leaves which were treated with a surfactant solution in some places is comparably intensive; at other positions their fluorescence is weaker, however, than in the case of transfersome-treated leaves.

The leaves which were treated with standard lipid vesicles do not fluoresce. Over large surface areas they are indistinguishable from the non-treated leaf regions.

This shows that transfersomes can transfer lipophilic substances spontaneously and irreversibly into a plant leaf or its surface. Their penetration capacity exceeds that of preparations containing highly concentrated surfactants, i.e. well established 'membrane fluidizers'.

Examples 143-145:

Composition:

50 mg; 43,5mg; 17,1 mg phosphatidylcholine from soy-bean
0.5 mg phosphatidylethanolamine-N-fluorescein
0 mg; 4,7 mg; 32,9 mg desoxycholate, Na-salt, p.a.
5 ml Hepes-buffer, pH 7,3

Preparation and results:

The preparation and results are identical with those of experiments 140-142.

Examples 146-148:Composition:

50 mg; 36,4; 20 mg phosphatidylcholine from soy-bean
0.5 mg phosphatidylethanolamine-N-fluorescein
0 mg; 13,6 mg; 30 mg Brij 35
5 ml Water

Preparation and results:

Preparation and results are comparable to those of experiments 140-142 and 143-145.

Examples 146-150:Composition:

84,2 to 25 mg phosphatidylcholine from soy-bean 80 %
75 kBq Giberellin A4, 3H-labelled
15,8 to 75 mg polyoxyethylene (23)-laurylether (Brij 35)
1 ml water

ethanol, absolute

Preparation:

An ethanolic lipid solution (50 %) is mixed with a corresponding amount of an ethanolic solution of giberellin and suspended in 1 ml of water or in appropriate volumes of a surfactant suspension to obtain a total lipid concentration of 10 % and L/S ratios of 8/1, 4/1, 2/1, 1/1 and 1/2. The resulting (mixed) suspension is then briefly homogenized with the aid of ultrasound so that the mean vesicle size is always below 300 nm.

Carrier suspensions are distributed over the surface of 3 leaves of *Ficus Benjaminii*; there, they are permitted to dry for 6 hours. After subsequent intensive washing of each leaf surface with 5 ml of water per square centimetre and destaining with a peroxide solution, the radioactivity in the homogenized plant material is measured scintigraphically in a beta-counter.

Agent transport in plant leaves:

Experiments show, as in examples 140-142, that transfersomes can bring the agent molecules into a leaf surface much more effectively than a micellar solution.

Examples 151-157:

Composition:

32,8-0.64 mg	phosphatidylcholine from soy-bean (purity higher than 95 %, PC)
75 kBq	dipalmitoylphosphatidylcholine tritium- labelled

2,2-34,4 mg	bile acid, Na-salt, p.a.
0.32 ml	phosphate buffer, pH 7,3

Preparation:

In each case, 35 mg of lipid are mixed with tritium-labelled dipalmitoylphosphatidylcholine in chloroform. After thorough drying under vacuum, the resulting mixture is suspended in 0.32 ml of buffer; the nominal surfactant/lipid ratios are 0; 0.125; 0.167; 0.263; 0.5 and 1 mol/mol. All suspensions are ultrasonicated until they are comparably opalescent, with the exception of the last, optically clear micellar solution. (The time for efficient necessary sonication decreases with increasing S/L). Control measurements with non-radioactive suspensions indicate that the mean 'particle' size in all samples must be around 100 nm. In all experiments approximately 1 day old suspensions are used.

Penetration into and through the intact skin:

On the back of an immobilized nude-mouse anaesthetized with ether six areas of 1x1 cm are marked. Each of these areas is covered with 20 microliters of a carrier suspension at 3x5 minutes intervals. 60 minutes later, the mouse is killed. From each treated area a sample is excised which is then cut to pieces, solubilized and de-stained. The skin-associated radioactivity is measured scintigraphically.

The corresponding results are summarized in figure 10. For comparison, the normalized values are also given which were taken from our patent application pertaining to the use of liposomes for topical anaesthesia. Optimal transfersomes are appreciably better than non-optimal preparations containing surfactants.

Examples 158-162:

Composition:

31 mg	phosphatidylcholine from soy-bean (purity higher than 95 %, PC)
75 kBq	dipalmitoylphosphatidylcholine tritium- labelled
4 mg	deoxycholate, Na-salt, p.a.
0.32 ml	phosphate buffer, pH 7,3

Preparation:

In each case 35 mg of lipid (PC and deoxycholate) are mixed with tritium-labelled dipalmitoylphosphatidylcholine in a chloroform solution. The resulting lipid mixture is dried and then dissolved in 30 microliters of warm, absolute ethanol. This solution is then mixed with 0.32 ml of a buffer solution (phosphate buffer, 10 mM, 0.9 % NaCl); this corresponds to a lipid/surfactant ratio of 4/1. The resulting suspension is thoroughly mixed and subsequently filtered through filters with pore sizes of 0.8; 0.45; 0.22 and 0.1 micrometers; this gives rise to vesicles with diameters of approx. 800, 400, 200 or 100 nm (suspensions A, B, C, D).

Penetration into and through the skin:

Tails of 2 anaesthetized mice are treated with 50 microlitres of a corresponding vesicle suspension for 15 minutes. Two control animals obtain an i.v. injection of 0.2 ml 1/10 diluted suspension B. After 30, 60, 120, 180, 240 and 360 minutes, blood specimens are drawn from the tail-tip. The radioactivity of these samples, which is determined by means of beta-scintigraphy, is a reliable indication of the systemic concentration of carrier-associated, radioactively labelled

lipids.

Experimental data show (fig. 11) that systemically applied transfersomes are eliminated from blood comparably as rapidly as standard liposomes. The size of carrier particles appears not to affect the spontaneous penetration into skin. All transfersomes investigated in this study can penetrate intact skin and get into the depth of a body quite effectively within a period of 4 hours at approx. 1 carrier; tendency increasing.

Examples 163-165:

Composition:

88 mg	phosphatidylcholine from soy-bean (purity higher than 95 %, PC)
75 kBq	inulin, tritium labelled
12 mg	deoxycholate, Na-salt, p.a.
100 ml	ethanol, absolute
0.9 ml	isotonic salt solution

Preparation:

100 mg of PC dissolved in 100 ml of warm ethanol, or a corresponding PC/deoxycholate solution ($L/S = 4.5$), are mixed with 0.9 ml of an isotonic salt solution (suspensions A and B, respectively). Each suspension is ultrasonicated until the mean vesicle size is about 150 nm.

12 microlitres of an aqueous solution of tritium-labelled inulin are pipetted into 38 microliters of a freshly prepared suspension of empty liposomes (A) or transfersomes (B). Subsequently, all mixtures are sonicated in closed vials for 60 minutes in an ultrasound bath at room temperature; they are all used for experiments within 24 after vesicle preparation.

Spontaneous inulin transfer through the skin:

On the abdomen of NMRI-mice in general anaesthesia, which three days before were depilated using medical tweezers, 10 microlitres of a vesicle suspension containing inulin in every case are applied twice at time intervals of approx. 3-5 minutes.

15, 30, 60, 120, 180, 240, 300 and 360 minutes later, 0.05 ml of blood are routinely taken from the tail of a each mouse to be then investigated scintigraphically. 6 hours later the subcutaneous tissues at the application site, as well as liver and spleen of all animals of this experiment are collected. After solubilization and decolouring procedures, these organs are also checked scintigraphically.

The results of this study are collected in figure 12. They show that normal liposomes can hardly mediate a percutaneous inulin uptake; in contrast to this, 6 hours later approx. 1.4 % of this marker which was applied in the form of transfersomes are found in the blood. This transfer sets in approximately 2-3 hours after the application and is not yet completed 6 hours after each application.

After 6 hours in the case of transfersomes, an average of 0.8 % (this corresponds to 24.1 % of the recovered dose) are in the skin at the application site; 0.9 % are found in the liver; spleen contains less than 0.1 % of the absolute dose. In the body (blood, spleen, liver) approximately 73.8 % of the recovered dose are thus found again.

In contrast to this, approximately 2 % of the normal liposomes at the application site can be detected by eye, the corresponding doses in the liver and spleen being below

0.1 %. This corresponds to a recovery of 95.3 % at the application site and 6.7 % of this dose in the body of the test animal.

Example 166:

Composition:

386 mg	phosphatidylcholine from soy-bean (purity > 95 %)
58.5 mg	sodium-cholate (L/S = 3,5)
500 µl	ethanol (96 %)
2.25 ml	0.9 % NaCl solution (per inject.)
2.25 ml	Actrapid HM™ 40 (corresponds to 90 I.U. of recombinant human insulin)

Preparation:

Samples are prepared essentially as described in examples 62-75. A mixture of aqueous salt solution and human recombinant insulin (with 6.75 mg m-cresole) is mixed with a lipid solution in ethanol. The resulting, opaque suspension is aged over night. 12 hours later, this suspension is pressed through a sterile filter (Anodisc™, pore diameter 0.2 micrometers) with the aid of nitrogen gas with excess pressure of 0.25 MPa under sterile conditions to be then filled into the glass container.

The nominal lipid/surfactant ratio is 3.5; the calculated molar surfactant concentration in the lipid double layer is approx. 5/1. This corresponds to 50 % of the concentration required for solubilization.

The mean radius of vesicles in final suspension in this experiment was 97 nm.

Application:

0.5 ml of a fresh, insulin containing transfersome suspension are applied onto the untreated skin of the left forearm of an informed, healthy male volunteer aged 37 years (starved for 18 hours) and distributed over an area of approx. 10 cm^2 . 5 minutes later, additional 300 microlitres of identical suspension are positioned in two halves on the forearm and upper arm, respectively. 5-10 minutes later, the suspension on the upper arm (dose approx. 2.5 mg/cm^2) has almost completely disappeared; it has thus nearly completely penetrated into skin. In contrast to this, lipids applied onto the forearm (dose approx. 7.5 mg/cm^2) are still well perceptible.

Activity:

In order to assess the biological activity of insulin, approx. 2 hours before the sample application, a permanent, soft catheter is placed into a vein in the right hand. Every 15-45 minutes, 1-1.5 ml of blood are collected from this catheter; the first 0.5-1 ml thereof are discarded; the remaining 0.5 ml are measured with a standard enzymatic glucose test. In each case three determinations with three to four independent specimens are made. The corresponding experimental data is summarized in figure 13. It shows that transfersomes mediate a significant hypoglycemia in the peripheral blood some 90 minutes after the drug application; this effect lasts for approx. 2 hours and amounts to approx. 50 % of the magnitude of the hypoglycemic effect of a comparable dose of subcutaneously applied insulin; the effect of the former lasts 200 % longer, however.

Examples 167-172:

Composition:

956 mg	phosphatidylcholine from soy-bean (+95 %)
0-26 mg	sodium-deoxycholate
1 mg	prostaglandine E1
1 ml	ethanol absolute
50 ml	0.9 % NaCl solution (per inject.)

Preparation:

1 ml of ethanol is pipetted into a glass flask with 1 mg of prostaglandine. After thorough mixing, the resulting prostaglandine solution is transferred to the appropriate amount of dry lipid in another glass vial. The original flask is flushed once again with the new lipid/prostaglandine solution and subsequently supplemented with 6 ml of an isotonic salt solution. The prostaglandine containing flask is washed twice with 2 ml of 0.9 % NaCl and mixed with the original lipid suspension. The sample is then divided into 5 parts; into individual aliquots sodium-desoxycholate is added at concentrations of 0; 1.6; 3.25; 6.5 or twice 13 mg/ml.

The resulting 10 % suspensions are aged for 24 hours. Subsequently they are either ultrasonicated or filtered manually through a 0.2 micrometer-filter, depending on cholate concentration. The specimens with the highest surfactant concentration are either filtered or ultrasonicated. Finally, the samples are diluted to obtain a final PGE1 concentration of 20 micrograms/ml and kept in dark glass bottles in a refrigerator. Vesicle radius right after sample preparation is 85 nm, two months later 100 nm.

Application and Action:

In each experiment 0.25 ml of a lipid suspension are applied on neighbouring but not interconnected regions of abdominal skin. 10 minutes later the skin surface is macroscopically dry; 15 minutes later, some of the application sites show a reddish appearance which, according to the test person's statement, is associated with a weak local pain. The intensity of oedema grades as 0, 0, 0, 0-1, 3 and 3 points (on a scale from 1-10).

This shows that merely transfersomes - but not liposomes or sub-optimal surfactant-containing vesicles - can penetrate into intact skin and thereby transfer drugs into body. The precise mode of sample preparation plays no role in this.

Examples 173-175:

Composition:

79.4 mg; 88.5 mg	phosphatidylcholine from soy-bean (+95%)
20.6 mg, 11.5 mg	sodium-deoxycholate
10 µg	hydrocortison
0.1 ml	ethanol absolute
1 ml	phosphate buffer, physiological

Preparation:

Lipids and hydrocortison are mixed as approx. 50 % ethanolic solution and subsequently supplemented with 0.95 ml of phosphate buffer. The resulting, very heterogeneous suspension is treated with ultrasound (25 W, 3-5 min). Specimens with an L/S ratio of 2/1 can be homogenized with ease, specimens with L/S = 4/1 are relatively difficult to homogenize.

Specimens with 1 and 2.5 weight-% result in stable suspensions

independent of the precise L/S ratio; 10 weight-% of agent cannot be incorporated into stable transfersomes of the given composition.

Examples 175-200:

Composition:

1.1 - 2mg phosphatidylcholine from soy-bean (+95%=PC)
0 - 32.5 mol-% Tween 80
pH 7.2 isotonic phosphate buffer

Preparation:

Different amounts of phospholipid and surfactant in each experiment are weighed or pipetted into 25 ml of buffer at ratios which yield suspensions with 0 - 32,5 mol-% of Tween 80 and a constant total lipid concentration of 2 %. Specimens are sterilized by filtering, filled into sterile glass vials and aged for 4 through 34 days. Then, the optical density of each sample is determined. This depends strongly on surfactant concentration but hardly on time within the framework of measuring conditions.

Characterization:

23 specimens each containing 3 ml of an individual lipid suspension are ultrasonicated in closed vials in a bath sonicator. Three, four and six hours later the samples' optical density is determined. Such measurements are repeated with every new sample series after the relative sample positions were exchanged in a systematic manner; the determination of optical density, again, is performed three, four and six hours after the start of sonication. All values corresponding to one concentration are summed up and divided

by the number of measurements; the resulting value is a measure of the samples' capacity for vesicularization under given conditions.

This procedure is an alternative or a supplement to the permeation resistance measurements as described in examples 40-49. Figure 16 shows, for example, that the amount of surfactant required for good mechanical deformability in the case of Tween 80 is 2-3 times lower than the corresponding solubilization concentration. This result is in good accord with the results of the permeation experiments.

Examples 201-215

Composition:

256.4-447 mg	phosphatidylcholine from soy-bean (+95% PC)
243.6-53.1 mg	Brij 96
0.26-0.45 ml	ethanol, absolute
4.5 ml	phosphate buffer, pH 6,5, 10 mM

Preparation:

Increasing volumes of Brij 96 are pipetted into the corresponding volumes of an alcoholic PC solution. Thus, a concentration series is obtained with L/S values between 1/1 and 1/8. After the addition of a buffer very heterogeneous liposomes are formed which are homogenized by means of filtering through a 0.2 μ m filter.

Permeation and carrier characteristics:

The already described method for the determination of suspensions permeability resistance is used. Corresponding values are given in the left panel of figure 14 as circles or

crosses (two independent test series). The functional dependence of the samples' permeability resistance as a function of the L/S ratio is similar to that of comparable transfersomes and is illustrated in the right panel of figure 14. The maximum permeation capacity is not reached before the L/S-value is below 3.

Examples 216-235

Composition:

202,0-413 mg	phosphatidylcholine from soy-bean (+95%PC)
298,0-87,0 mg	Myrj 49
0.26-0.45 ml	ethanol, absolute
4.5 ml	phosphate buffer, pH 6,5, 10 mM

Preparation and Characterization:

Transfersomes are made and characterized as described for examples 201-215. Their permeation properties as a function of the relative surfactant concentration in the individual specimen is given in the left panel of figure 15. The right panel gives corresponding equilibrium values; the latter, however, provide no information about vesicle suitability for permeation and agent transport.

Example 236:

Composition:

144,9 mg	phosphatidylcholine from soy-bean
24.8 mg	desoxycholate, Na-salt
1.45 ml	Actrapid HM 100 (145 I.U.)
0.16 ml	ethanol, absolute

Preparation:

Appropriate quantities of both lipids are dissolved in corresponding amounts of ethanol and mixed with a standard solution of insulin. 12 hours later, the crude carrier suspension is homogenized by means of filtration. Average vesicle diameter is 225 ± 61 nm and nominal insulin concentration is 83 I.U. Over an area of appr. 10 square centimeters on the right forearm 0.36 ml (30 I.U.) of insulin in transfersomes are distributed. Blood samples are taken every 10 minutes through a heparinized soft catheter positioned in a vein in the right forearm; the first 0.5 ml are always discarded; the following 0.5-0.8 ml of each sample are sedimented and immediately frozen; the remainder of each sample is used for the determination of blood glucose concentration during the experiment.

Activity:

These liposomes with a relatively high surfactant concentration have only a very limited capability of transporting insulin across skin, as is seen from figure 17. Depending on the choice of data used for evaluation, the lowering of the blood glucose level does not exceed 2 to 5 mg/dl over a period of 30-40 minutes at the most. The effect of a comparable subcutaneous injection is 50 to 200 times higher. Surfactant-containing liposomes, which have not been optimized with regard to their 'transfersomal' properties, are consequently poorly suited for the use as carriers in the case of dermal applications. Surfactant concentration in such carriers thus cannot mediate an optimal agent permeation through skin.

This shows that formulations prepared according to this invention can (still) have a partial activity even if their

content of edge active substances has not been optimized; however, a maximum advantage can only be achieved after the concentration of an edge active substance requiring maximum permeation has been determined and used as described in this patent application.

Possible use of transfersomes for the application of antidiabetics, most notably of insulin, which has been discussed above in examples 166 and 236, will be investigated in more detail in the following text.

Attempts to bring antidiabetic agents into a body without the use of an injection needle have been known for quite some time already (see, for example, the review article by Lassmann-Vague, *Diabete. Metab.* 14, 728, 1989). It has been proposed, for example, to use implantable insulin containers (Wang, P.Y, *Biomaterials* 10. 197, 1989) or pumps (Walter, H et al., *Klin. Wochenschr.* 67, 583, 1989), to administer an insulin solution transnasally (Mishima et al., *J. Pharmacobio.-Dynam.* 12, 31, 1989), perocularly (Chiou et al., *J. Ocul. Pharmacol.* 5, 81, 1989), perorally in a liposomes suspension (Rowland & Woodley, *Biosc. Rep.* 1, 345, 1981) or transrectally; in order to introduce insulin molecules through the skin, a corresponding solution was jet-injected (Siddiqui & Chies, *Crit. Rev. Ther. Drug. Carrier. Syst.* 3, 195, 1987), or brought through the skin with the aid of small injectors (Fiskes, *Lancet* 1, 787, 1989), electric fields (Burnette & Ongpipattanakul, *J. Pharm. Sci.* 76, 765, 1987; Meyer, B.R et al., *Amer. J. Med. Sci.* 297, 321, 1989); chemical additives should also support drug permeation.

All these procedures have hardly brought any real improvements for the therapy of diabetes patients - with the exception of jet injection, perhaps; but the latter is only a very refined, technically extremely complicated form of injection and,

consequently, not very common. The daily therapy of each insulin-dependent patient, consequently, still involves injecting an insulin solution under the skin or into the muscle tissue (De Meijer, P. et al., *Neth. J Med.* 34, 210. 1989).

Lipids have thus far been discussed as excipients for delayed insulin release in insulin implants (Wang, P.Y *Int. J Pharm.* 54, 223, 1989); in the form of liposomes they were also suggested for use as vehicles for peroral applications (Patel, 1970), without the therapeutic results really being reproducible, however, (*Biochem. Int.* 16, 983, 1988). Subsequent publications in the field of insulin containing liposomes, therefore, have dealt with methodological rather than therapeutic issues (Wiessner, J. H. and Hwang, K. J. *Biochim. Biophys. Acta* 689, 490 1982; Sarrach, D. *Stud. Biophys.* 100. 95, 1984; Sarrach, D. and Lachmann, U. *Pharmazie* 40. 642, 1985; Weingarten, C. et al., *Int. J. Pharm.* 26, 251, 1985; Sammins, M.C. et al., *J. Pharm. Sci.* 75, 838, 1986; Cervato, G. et al., *Chem. Phys. lipids* 43, 135, 1987).

According to this invention, the transfersomes described above are used for non-invasive applications of antidiabetic agents, most frequently of insulin, in formulations which were optimized for this purpose.

It is advantageous to use at least one carrier substance for this purpose from the class of physiologically tolerable polar or non-polar lipids or some other pharmacologically acceptable amphiphiles; well-suited molecules are characterized by their ability to form stable agent carrying aggregates. The preferred aggregate form are lipid vesicles, the most preferred membrane structure is a lipid double layer.

It is, furthermore, considered advantageous if at least one

such substance is a lipid or a lipoid from a biological source or some corresponding synthetic lipid; or else, a modification of such lipids, for example a glyceride, glycerophospholipid, sphingolipid, isoprenoidlipid, steroid, sterine or sterol, a sulfur- or carbohydrate-containing lipid, or any other lipid which forms stable double layers; for example, a half-protonated fluid fatty acid. Lipids from eggs, soy-bean, coconuts, olives, safflower, sunflower, linseed, whale oil, Nachtkerze or primrose oil, etc. can be used, for example, with natural, partly or completely hydrogenated or exchanged chains. Particularly frequently, the corresponding phosphatidylcholines are used; as well as phosphatidyl-ethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidic acids and phosphatidylserines, sphingomyelins or sphingophospholipids, glycosphingolipids (e.g. cerebrosides, ceramidpolyhexosides, sulfateids, sphingoplasmalogenes); gangliosides or other glycolipids are also suitable for the use in transfersomes according to this invention. Amongst the synthetic lipids especially the corresponding dioleoyl-, dilinoleyl-, dilinolenyl-, dilinolenoyl-, diaracidonyl-, dimyristoyl-, less frequently dipalmitoyl-, distearoyl-, phospholipide or the corresponding sphingosin derivatives, glycolipids or other diacyl- or dialkyl-lipids are used; arbitrary combinations of the above-mentioned substances are also useful.

It is advantageous if an edge active substance is a nonionic, a zwitterionic, an anionic or a cationic surfactant. It can also contain an alcohol residue; quite suitable components are long-chain fatty acids or fatty alcohols, alkyl-trimethyl-ammonium-salts, alkylsulfate-salts, cholate-, deoxycholate-, glycodeoxycholate-, taurodeoxycholate-salts, dodecyl-dimethyl-aminoxide, decanoyl- or dodecanoyl-N-methylglucamide (MEGA 10, MEGA 12), N-dodecyl-N,N-dimethylglycine, 3-(hexadecyldimethylammonio)-propanesulfonate, N-hexadecyl-

sulfobetaine, nonaethyleneglycol-octylphenylether, nonaethylene-dodecylether, octaethyleneglycol-isotridecylether, octaethylene-dodecylether, polyethylene glycol-20-sorbitane-monolaurate (Tween 20), polyethylene glycol-20-sorbitane-monooleate (Tween 80), polyhydroxyethylene-cetylstearylether (Cetomacrogol, Cremophor O, Emulgin, C 1000) polyhydroxyethylene-4-laurylether (Brij 30), polyhydroxyethylene-23-laurylether (Brij 35), polyhydroxyethylene-8-stearate (Myrj 45, Cremophor AP), polyhydroxyethylene-40-stearate (Myrj 52), polyhydroxyethylene-100-stearate (Myrj 59), polyethoxylated castor oil 40 (Cremophor EL), polyethoxylated hydrated castor oil, sorbitane-monolaurate (Arlacel 20, Span 20), especially preferred decanoyl- or dodecanoyl-N-methylglucamide, lauryl- or oleoylsulfate-salts, sodiumdeoxycholate, sodiumglycodeoxycholate, sodiumoleate, sodiumelaidate, sodiumlinoleate, sodiumlaurate, nonaethylene-dodecylether, polyethylene-glycol-20-sorbitane-monooleate (Tween 80), polyhydroxyethylene-23-lauryl ether (Brij 35), polyhydroxyethylene-40-stearate (Myrj 52), sorbitane-monolaurate (Arlacel 20, Span 20) etc.

Amongst the most suitable surfactants in these classes of substances are: n-tetradecyl(=myristoyl)-glycero-phosphatidic acid, n-hexadecyl(=palmityl)-glycero-phosphatidic acid, n-octadecyl(=stearyl)-glycero-phosphatidic acid, n-hexadecylene(=palmitoleil)-glycero-phosphatidic acid, n-octadecylene(=oleil)-glycero-phosphatidic acid, n-tetradecyl-glycero-phosphoglycerol, n-hexadecyl-glycero-phosphoglycerol, n-octadecyl-glycero-phosphoglycerol, n-hexadecylene-glycero-phosphoglycerol, n-octadecylene-glycero-phosphoglycerol, n-tetradecyl-glycero-phosphoserine, n-hexadecyl-glycero-phosphoserine, n-octadecyl-glycero-phosphoserine, n-hexadecylene-glycero-phosphoserine and n-octadecylene-glycero-phosphoserine.

Total concentration of the basic carrier substance is normally between 0.1 and 30 weight-%; preferably, concentrations between 0.1 and 15 %, most frequently between 5 and 10 % are used.

Total concentration of the edge active substance in the system amounts to 0.1 % through to 99 mol-% of the quantity which is required to solubilize the carrier, depending on each application. Frequently, the optimum is drug dependent - in a concentration range between 1 and 80 mol-%, in particular between 10 and 60 mol-%; most frequently values between 20 and 50 mol-% are favoured.

The concentration of the drug agent in the case of insulin is most frequently in the range between 1 and 500 I.U./ml; concentrations between 20 and 100 I.U./ml are preferred; carrier concentration in the latter case is in the range between 0.1-20 weight-%, frequently between 0.5 and 15 weight-%, most frequently between 2.5 and 10 weight-%.

For preparing a therapeutic formulation, the carrier substances, which are very frequently lipids, are taken as such or dissolved in a physiologically acceptable solvent or a water-miscible solubilizing agent, combined with a polar solution, and made to form carriers.

It is advantageous to use polar solutions containing edge active substances; the latter can also be used with lipids or be contained in a lipid solution.

Carrier formation is preferably initiated by stirring in, by means of evaporation from a reverse phase, by means of an injection or a dialysis procedure, through mechanical agitation, such as shaking, stirring, homogenization,

ultrasonication, friction, shear, freezing-and-thawing, by means of high-and low-pressure filtration, or any other use of energy.

It may be advantageous to incorporate agents only after carrier formation.

If transfersomes are prepared by means of filtration, materials with a pore size of 0.1-0.8 micrometers, very frequently of 0.15-0.3 micrometers, and particularly preferred of 0.22 micrometers are preferably used; several filters can also be used in combination or in a row.

In the case that transfersomes are made by means of ultrasonication, energy densities in the order of 10-50 kW/litre/minute are preferably used; in stirring or rotary machines 1,000 through to 5,000 revolutions per minute are typically used. If high pressure homogenizers are used, pressures in the order of 300-900 Bar normally ensure sufficient transfersome homogeneity and quality after a single passage; in the latter case even suspensions with 20-30 % lipids can be processed without any difficulty.

It is often sensible to prepare transfersomes only shortly before an application from a concentrate or lyophilisate.

Cryopreservatives, such as oligosaccharides, can facilitate the formation of transfersomes from a lyophilisate.

Standard agent, supporting, or additional substances, in particular the stabilizing, protective, gel-forming, appearance-affecting substances and markers can also be used as described in this application.

The following examples illustrate this invention without

implying any limits to its general use. Temperatures are given in degree Celsius, carrier sizes in nanometers, and other quantities in common SI units.

Example 237:

Composition:

120 mg	phosphatidylcholine from soy-bean (purity > 95 %)
20 mg	sodium-cholate p.a. (L/D = 3,2)
150 µl	ethanol (96 %)
1.45 ml	Actrapid HM 100 (recombinant human insulin 100 I.U./ml)

Preparation:

This preparation is produced as described in example 166, with only minor modifications. The main difference is that the lipid/insulin mixture is hand-filtered through a 0.22 µm polycarbonate filter (Sartorius) using a 1 ml injection already few minutes after mixture preparation. The final volume of the suspension is 1.2 ml; the nominal lipid/cholate ratio is 2.8/1, in lipid membranes approx. 2.4/1. The final concentration of insulin is approx. 83 I.U./ml; the vesicle radius one day after preparation is 94 nm on the average; one week later, 170 nm.

Application:

One and half hours after the beginning of the experiment, 240 µl of a sterile suspension of insulin containing transfersomes (with 20 I.U.) were taken. These were applied and uniformly smeared at a dose of approx. 0.7 mg lipid/cm² over the inner side of the right forearm of a male test person starved for 18

hours prior to experiment. 5 minutes later the skin surface is macroscopically dry. Another 45 minutes later no traces of application are visible anymore.

Activity:

At irregular intervals of between 15 and 40 minutes, blood samples are drawn from a soft i.v. catheter placed in the left forearm. The determination of the blood glucose level is performed as described in example 166.

The course in time of the transfersome mediated hypoglycemia is represented in figure 18. The blood glucose level decreases approx. 1.5 hours after drug application by some 10 mg/ml; this artificial hypoglycemia lasts for 4 hours at least and thus attains 70-80 % of the value which can be achieved by a subcutaneous application of a comparable amount of the drug Actrapid. The results of control experiments in which the insulin containing transfersomes are injected subcutaneously are shown as crosses in this figure. The total effect in the latter case is similar to that induced by the free drug injected s.c.

Example 238:

Composition:

216 mg	phosphatidylcholine from soy-bean (487 μ l of a 50 % solution in absolute ethanol)
27 mg	phosphatidylglycerol from egg (98 %)
29.45 mg	oleic acid, puriss.
3 ml	Actrapid HM 100 (recombinant human insulin 100 I.U./ml)
40 μ l	1 N NaOH
20 μ l	1 N NaCl

Preparation:

Lipids are mixed until solution is homogeneously clear. After the addition of an actrapid solution, of alkali and salt solution, an optically opalescent suspension is formed. Filtering of this suspension through a polycarbonate filter with a pore diameter of $0.2 \mu\text{m}$ yields a much less opalescent suspension which consists of vesicles (transfersomes) with a mean diameter of 320 nm.

Application:

Starting glucose concentration in the blood of a test person (70 kg, 37 years, normoglycemic, starved for 24 hours) is measured over a period of 90 minutes for reference. Subsequently, the above-mentioned transfersome suspension with a nominal concentration of 85 I.U. insulin/ml, which has been aged for 12 hours at 4°C , is applied on the right forearm skin (approx. $330 \mu\text{l}$ over an area of approx. 15 cm^2); this corresponds to a total applied dose of 28 I.U.

Activity:

Blood specimens are collected through a heparinized, permanent, soft catheter placed in a vein in the left forearm; 0.5 ml of each sample are sedimented and immediately frozen for further use. The remaining volume is used for the in situ determination of the blood glucose concentration by an enzymatic method. The measured glucose concentration decreases by approx. 8 mg/dl after approx. 2.5 hours and remains diminished for more than 4.4 hours. This corresponds to 75 % of the maximally achievable effect, as concluded from control experiments performed by injecting insulin s.c. The pharmacokinetics of this experimental series is represented in

figure 19.

Figure 20 gives the results of three typical experiments with insulin. They illustrate the results obtained by one percutaneous and two s.c. drug applications.

Example 239:

Composition:

143 mg	phosphatidylcholine from soy-bean
18 mg	phosphatidylglycerol from egg (98 %)
19.6 mg	oleic acid, puriss.
2 ml	Actrapid HM 100 (200 I.U.)
25 μ l	1 N NaOH

Preparation:

Lipids are weighed into a glass vial and mixed with a standard insulin solution. The resulting opaque suspension is ultrasonicated directly, using a titanium probe-tip (approx. 5 W, 3x5 seconds at 22°C in 60 seconds intervals). The resulting, optically clear but still opalescent suspension contains vesicles with a mean radius of 114 ± 17 nm.

Application and Activity:

The results of this test series are within the limits of experimental error identical to those obtained in example 238.

Example 240:

Composition:

143 mg	phosphatidylcholine from soy-bean
--------	-----------------------------------

18 mg	phosphatidylglycerol from egg (98 %)
20.5 mg	sodium oleate
2 ml	Actrapid HM 100 (200 I.U.)

Preparation:

The lipids are dissolved in a glass vial in 0.15 ml abs. ethanol and then combined with a standard insulin solution. Further procedure is as described in example 239.

Application and Activity:

Over an area of approx. 5 cm² on the forearm skin of a test person a piece of fine-mesh synthetic cloth is fixed. This is then covered with 350 µl of an insulin containing transfersome suspension and left uncovered to dry.

The resulting decrease of the blood glucose level after 4 hours amounts to 7.8 mg/dl and after 6 hours to 8.5 mg/dl. It is thus comparable to the result obtained in experiment no. 238.

Example 241:

The procedure is at first as described in example 238 except that no salt solution is added to the sample suspension; the opaque crude transfersome suspension is divided into two parts. One of these consisting of 50 % of the total volume is passed through a sterile filter; the other half is ultrasonicated for 15 seconds at room temperature at a power of approx. 5 W. The mean diameter of carriers in both halves is similar, 300 nm or 240 nm, respectively.

Example 242:

The procedure is as described in examples 238 and 240. Transfersomes, however, are filtered one, two and three times in a row. The mean vesicle diameter in the resulting three samples are 300, 240, and 200 nm, resp..

The transfersomes of examples 241 and 242 yield similar hypoglycemic results in biological tests as those of example 238.

Example 243:

Composition:

144,9;152 mg	phosphatidylcholine from soy-bean
24.8;17.6 mg	desoxycholate, Na-salt
1.45;1.55 ml	Actrapid HM 100 (145 I.U.)
0.16 ml	ethanol, absolute

Preparation:

Lipids are weighed into glass vials, dissolved with ethanol and mixed with an insulin solution. The resulting opaque suspension is aged over night and subsequently filtered through a 0.22 micrometer filter at t=12 hours. The nominal insulin concentration is 83 or 84 I.U; the mean vesicle radius in both cases is 112 nm.

Application and Activity:

General experimental conditions are as described in examples 237-239. Transfersome suspensions (0.36 ml, corresponds to 30 I.U.) are applied onto the inner side of a forearm skin in both cases; the blood samples are taken from a soft catheter placed in a vein in the other forearm.

The results of these two experiments are given in figure 21. They show that preparations with a relatively high surfactant concentration (Sample 1, $L/S=3/1$) can cause a hardly significant decrease in the blood glucose level; transfersomes close to their optimum, however, with a surfactant concentration lower by approx. 30 % ($L/S=4.5/1$), cause a very pronounced 'hypoglycemia' which lasts for many hours.

This is another proof that the transfersomes tend to transport drugs through intact skin according to a completely new principle of action which is dissimilar to that of classical pharmaceutical formulations.

This example, in addition to example 236, furthermore, suggests the following conclusion: for the systems investigated, also surfactant concentrations can be used which are remote from the transfersomal optimum (without the carrier activity being lost completely); notwithstanding this, particularly advantageous results are obtained when the surfactant concentration has been determined and chosen to be in a range which ensures maximum carrier elasticity and thus permeation capability of the transfersomes in combination with sufficiently high carrier stability to dissolution, bursting, agent loss, etc.

Claims:

1. A preparation for the transport of an agent through a permeability barrier, the preparation being in the form of minute droplets of fluid with a membrane-like coating consisting of one or several layers of amphiphilic molecules or of one amphiphilic carrier substance, wherein the preparation contains a surface-active substance in a concentration that amounts to up to 99 mol % of the concentration of such substance required for droplet solubilization whereby the amount of this substance approaches the solubilization point to an extent that conveys to the droplet a maximum permeation capability while simultaneously maintaining its stability.
2. A preparation according to claim 1, wherein the concentration of surface-active substance amounts to at least 0.1 mol % of the solubilization-inducing concentration of surface-active substances.
3. A preparation according to claim 1, wherein the concentration of surface-active substance amounts to between 1 and 80 mol % of the solubilization-inducing concentration of surface-active substances.
4. A preparation according to claim 1, wherein the concentration of surface-active substance amounts to between 10 and 60 mol % of the solubilization-inducing concentration of surface-active substances.
5. A preparation according to claim 1, wherein the concentration of surface-active substance amounts to between 20 and 50 mol % of the solubilization-inducing concentration of surface-active substances.

6. A preparation according to any one of claims 1 to 5, wherein the preparation contains an amount of an amphiphilic substance as a carrier or as a basis for the membrane-like coating of the droplet forming hydrophilic fluid, the agent being contained in the carrier substance, in the shell, and/or in the droplet material itself.
7. A preparation as claimed in claim 6, wherein said amphiphilic substance is a lipid-like material and said surface-active substance is a surfactant.
8. A preparation as claimed in any one of claims 1 to 7, wherein the content of said amphiphilic substance for the applications on human or animal skin amounts to 0.01 through 30 weight % of the preparation mass.
9. A preparation as claimed in any one of claims 1 to 7, wherein the content of said amphiphilic substance for the applications on human or animal skin amounts to between 0.1 and 15 weight % of the preparation mass.
10. A preparation as claimed in any one of claims 1 to 7, wherein the content of said amphiphilic substance for the applications on human or animal skin amounts to between 5 and 10 weight % of the preparation mass.
11. A preparation as claimed in any one of claims 1 to 7, wherein the content of the amphiphilic substance in the formulation for application on plants is 0.000001 through 10 weight %.

12. A preparation as claimed in any one of claims 1 to 7, wherein the content of the amphiphilic substance in the formulation for application on plants is between 0.001 and 1 weight %.
13. A preparation as claimed in any one of claims 1 to 7, wherein the content of the amphiphilic substance in the formulation for application on plants is between 0.01 and 0.1 weight %.
14. A preparation as claimed in any one of claims 1 to 13, wherein the agent is an adrenocorticostatic, a β -adrenolytic, an androgen or antiandrogen, antiparasitic, anabolic, anaesthetic or analgesic, analeptic, antiallergic, antiarrhythmic, antiarterosclerotic, antiasthmatic and/or bronchospasmolytic, antibiotic, antidepressant and/or antipsychotic, antidiabetic, an antidote, antiemetic, antiepileptic, antifibrinolytic, anticonvulsive, an anticholinergic, and enzyme, coenzyme or a corresponding inhibitor, an antihistaminic, antihypertonic, a biological inhibitor of drug activity, an antihypotonic, anticoagulant, antimycotic, antimyasthenic, an agent against Morbus Parkinson, an antiphlogistic, antipyretic, antirheumatic, antiseptic, a respiratory analeptic or a respiratory stimulant, a bronchohytolytic, cardiogenic, chemotherapeutic, a coronary dilator, a cytostatic, a diuretic, a ganglion-blocker, a glucocorticoid, an antifu agent, a haemostatic, hypnotic, an immunoglobuline or its fragment or any other immunologically active substance, a bioactive carbohydrate (derivative), a contraceptive, an antimigraine agent, a mineralcorticoid, a morphine-antagonist, a muscle relaxant, a narcotic, a neuraltherapeutic, a nucleotide, a neuroleptic, a neurotransmitter or some of its antagonists, a peptide

(derivative), an opthalmic, (para)-sympaticomimetic or (para)-sympathicolytic, a protein (derivative), a psoriasis/neurodermitis drug, a mydriatic, a psychostimulant, rhinologic, any sleep-inducing agent or its antagonist, a sedating agent, a spasmolytic, tuberlostatic, urologic, a vasoconstrictor or vasodilator, a virustatic or any wound-healing substance, or several such agents.

15. A preparation as claimed in any one of claims 1 to 11, wherein said agent is a growth modulating substance for living organisms.
16. A preparation as claimed in any one of claims 1 to 11, wherein said agent exerts biocidal activity.
17. A preparation as claimed in any one of claims 1 to 11, wherein said agent is an insecticide, a pesticide, a herbicide or a fungicide.
18. A preparation as claimed in any one of claims 1 to 11, wherein the agent is an attractant.
19. A preparation as claimed in any one of claims 1 to 11, wherein the agent is selected from the class of pheromones.
20. A method for manufacturing a preparation for the application of an agent in the form of minute droplets of a fluid, the preparation comprising a membrane-like "envelope" consisting of one or several layers of amphiphilic molecules, or supplemented with an amphiphilic carrier substance, for the transport of an agent in and through natural barriers and constrictions, wherein the concentration of a surface-active substance

required for the solubilization of a carrier entity is determined and then an amount of the surface-active substance which is close to the former concentration but still guarantees a sufficient carrier stability and permeation capability is used for the preparation.

21. A method according to claim 20, wherein the natural barrier is skin.
22. A method as claimed in claim 21, wherein the stability and the permeation capacity of the "droplet" are determined by means of filtration, if required under pressure, through a fine-pore filter or by means of any other controlled mechanical fragmentation.
23. A method as claimed in claim 20, 21 or 22, wherein the content of said surface-active substance is between 0.1 and 99 mol %, of the concentration at which solubilization of the carrier is achieved.
24. A method as claimed in claim 20, 21 or 22, wherein the content of said surface-active substance is between 1 and 80 mol %, of the concentration at which solubilization of the carrier is achieved.
25. A method as claimed in claim 20, 21 or 22, wherein the content of said surface-active substance is between 10 and 60 mol %, of the concentration at which solubilization of the carrier is achieved.
26. A method as claimed in claim 20, 21 or 22, wherein the content of said surface-active substance is between 20 and 50 mol %, of the concentration at which solubilization of the carrier is achieved.

27. A method as claimed in any one of claims 20 to 26, wherein the preparation is subjected to filtration, ultrasonication, stirring, agitating or any other mechanical fragmentation.
28. A preparation as claimed in any one of claims 1 to 19, wherein said preparation comprises at least one antidiabetic agent.
29. A preparation as claimed in any one of claims 1 to 19, wherein said preparation comprises insulin.
30. A preparation as claimed in claim 28 or 29, wherein it contains a physiologically compatible polar or non-polar lipid as an amphiphilic carrier substance, the carrier membrane having a double layer structure.
31. A preparation as claimed in claim 30, wherein the amphiphilic substance is a lipid or a lipoid from any biological source or a corresponding synthetic lipid, or else comprises a modification of such lipids, a glyceride, steroid, sterin or sterol, a sulfur- or carbohydrate-containing lipid, or any other lipid which forms stable double layers, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, a phosphatidic acid, a phosphatidylserin, a sphingomyelin or sphingophospholipid, a glycosphingolipid, a ganglioside or other glycolipid or a synthetic lipid, a dioleoyl-, dilinoleyl-, dilinolanyl-, dilinolenoyleyl-, diarachidoylel-, dimyristoylel-, dipalmitoylel-, distearoylel-, phospholipid or corresponding sphingosinderivative, a glycolipid or other diacyl- or dialkyl-lipid.

32. A preparation as claimed in claim 30, wherein the amphiphilic substance is selected from glycerophospholipid, isoprenoidlipid, sphingolipid, a half-protonated fluid fatty acid, cerebroside, ceramidepolyhexoside, sulfatide and sphingoplasmalogene.
33. A preparation as claimed in any one of claims 28 to 32, containing several surface-active substances.
34. A preparation as claimed in any one of claims 28 to 33, wherein said surface-active substance is a nonionic, a zwitterionic, an anionic or a cationic surfactant, a long-chain fatty acid or a long-chain fatty alcohol, an alkyl-trimethyl-ammonium-salt, alkylsulfate-salt, cholate, deoxycholate-, glycodeoxycholate-, taurodeoxycholate-salt, dodecyl-dimethyl-aminoxide, decanoyl- or dodecanoyl-N-methylglucamide, N-dodecyl-N, N-dimethylglycine, 3-(hexadecyldimethylammonio)-propane-sulfonate, N-hexadecyl-sulfobetaine, nonaethylene-glycol-octylphenylether, nonaethylene-dodecylether, octaethylene-glycol-isotridecylether, octaethylene-dodecylether, polyethylene glycol-20-sorbitane-monolaurate, polyethylene glycol-20-sorbitane-monooleate, polyhydroxyethylene-cetylstearyl ether, polyhydroxyethylene-4-laurylether, polyhydroxyethylene-23-laurylether, polyhydroxyethylene-8-stearate, polyhydroxyethylene-40-stearate, polyhydroxyethylene-100-stearate, polyethoxylated castor oil 40, polyethoxylated hydrated castor oil, sorbitane-monolaurate, particularly preferred decanoyl- or dodecanoyl-N-methylglucamide, lauryl- or oleoylsulfate-salts, sodiumdeoxycholate, sodiumglycodeoxycholate, sodiumoleate, sodiumelaiate, sodiumlinoleate, sodiumlaurate, nonaethylene-dodecylether, polyethylene glycol-20-sorbitane-monooleate, polyhydroxyethylene-23-laurylether,

polyhydroxyethylene-40-stearate and/or sorbitane-monolaurate and lysophosphipids, n-octadecylen(=oleoyl)-glycero-phosphatidic acid, -phosphorylglycerol, or -phosphoryl-serine, n-dilauryl-glycero-phosphatidic acid, -phosphoryl glycerol, or -phosphorylserine, n-tetradecyl-glycero-phosphatidic acid, -phosphorylglycerol, or -phosphorylserine and corresponding palmitoeloyl-, elaidoyl-, vaccenyl-lysophospholidids.

35. A preparation as claimed in any one of claims 28 to 34, comprising 1 through 500 I.U. insulin/ml as agent.
36. A preparation as claimed in any one of claims 28 to 34, comprising between about 20 and 100 I.U. insulin/ml.
37. A preparation as claimed in any one of claims 28 to 36, wherein the concentration of the carrier substance is in the range of about 0.1 to 20 wt %, based on the weight of the preparation.
38. A preparation as claimed in any one of claims 28 to 36, wherein the concentration of the carrier substance is in the range of about 0.5 to 15 wt %, based on the weight of the preparation.
39. A preparation as claimed in any one of claims 28 to 36, wherein the concentration of the carrier substance is in the range of about 2.5 to 10 wt %, based on the weight of the preparation.
40. A preparation as claimed in any one of claims 28 to 35, wherein a phosphatidylcholine and/or a phosphatidylglycol is used as an amphiphilic substance, and a lysophosphatidic acid or lysophosphoglycerol, a deoxycholate-, glycodeoxycholate- or cholate salt, a

laurate, myristate, oleate, plamitoleate, or a corresponding phosphate- or sulfate-salt, and/or a Tween- or a Myrj-surfactant is used as a surface-active substance.

41. A preparation according to claim 40, comprising human insulin as agent.
42. A preparation according to claim 41, wherein the insulin is recombinant insulin.
43. A preparation as claimed in any one of claims 28 to 42, wherein radius of the droplets is between about 50 and about 200 nm.
44. A preparation as claimed in any one of claims 28 to 42, wherein the radius of the droplets is between about 100 and about 180 nm.
45. A method for the preparation of a formulation for the non-invasive application of antidiabetic agents, wherein liposome-like droplets are formed from at least one amphiphilic substance, at least one hydrophilic substance, at least one surface-active substance, and at least one antidiabetic agent, comprising the steps of mixing together the surface-active substance and the amphiphilic substance, and separately mixing together the hydrophilic substance and the antidiabetic agent which can be, if required, dissolved in a solution, the resulting mixtures or solutions then being combined as one mixture to induce the formation of carrier particles.
46. A method as claimed in claim 45, wherein the mixtures or solutions are combined by action of mechanical energy.

47. A method as claimed in claim 45 or 46, wherein said amphiphilic substance is either used as such or dissolved in a physiologically compatible solvent which is very frequently miscible with hydrophilic fluids, or in a solvation mediating agent together with a polar solution.
48. A method as claimed in claim 47, wherein the physiological compatible solvent is miscible with water.
49. A method as claimed in claim 47 or 48, wherein the polar solution contains at least one surface-active substance.
50. A method as claimed in any one of claims 45 to 49, wherein formation of droplets is induced by substance addition into a fluid phase, evaporation from a reverse phase, using an injection- or dialysis procedure, with the aid of mechanical stress such as shaking, stirring, homogenizing, ultrasonication, shear, freezing and thawing, or high- or low-pressure filtration.
51. A method as claimed in claim 50, wherein formation of droplets is caused by filtration, the filtering material having pore diameters of 0.1 through 0.8 μm .
52. A method as claimed in claim 51, wherein the filtering material has a pore diameter of about 0.15 to 0.3 μm .
53. A method as claimed in claim 51, wherein the filtering material has a pore diameter of about 0.22 μm .
54. A method as claimed in claim 51, 52 or 53, wherein several filters are used in sequence.

55. A method as claimed in any one of claims 45 to 54, wherein inclusion of the agent occurs at least partly after the droplet formation.
56. A method as claimed in any one of claims 45 to 55, wherein liposome-like droplets are prepared just before their application from a suitable concentrate or a lyophilisate.
57. A preparation according to claim 2, wherein the activity of a droplet unit is about 10 Piconewtons or less.
58. A preparation according to claim 3, wherein the activity of a droplet unit is about 10 Piconewtons or less.
59. A preparation according to claim 4, wherein the activity of a droplet unit is about 10 Piconewtons or less.
60. A preparation according to claim 5, wherein the activity of a droplet unit is about 10 Piconewtons or less.

FIGURE 1

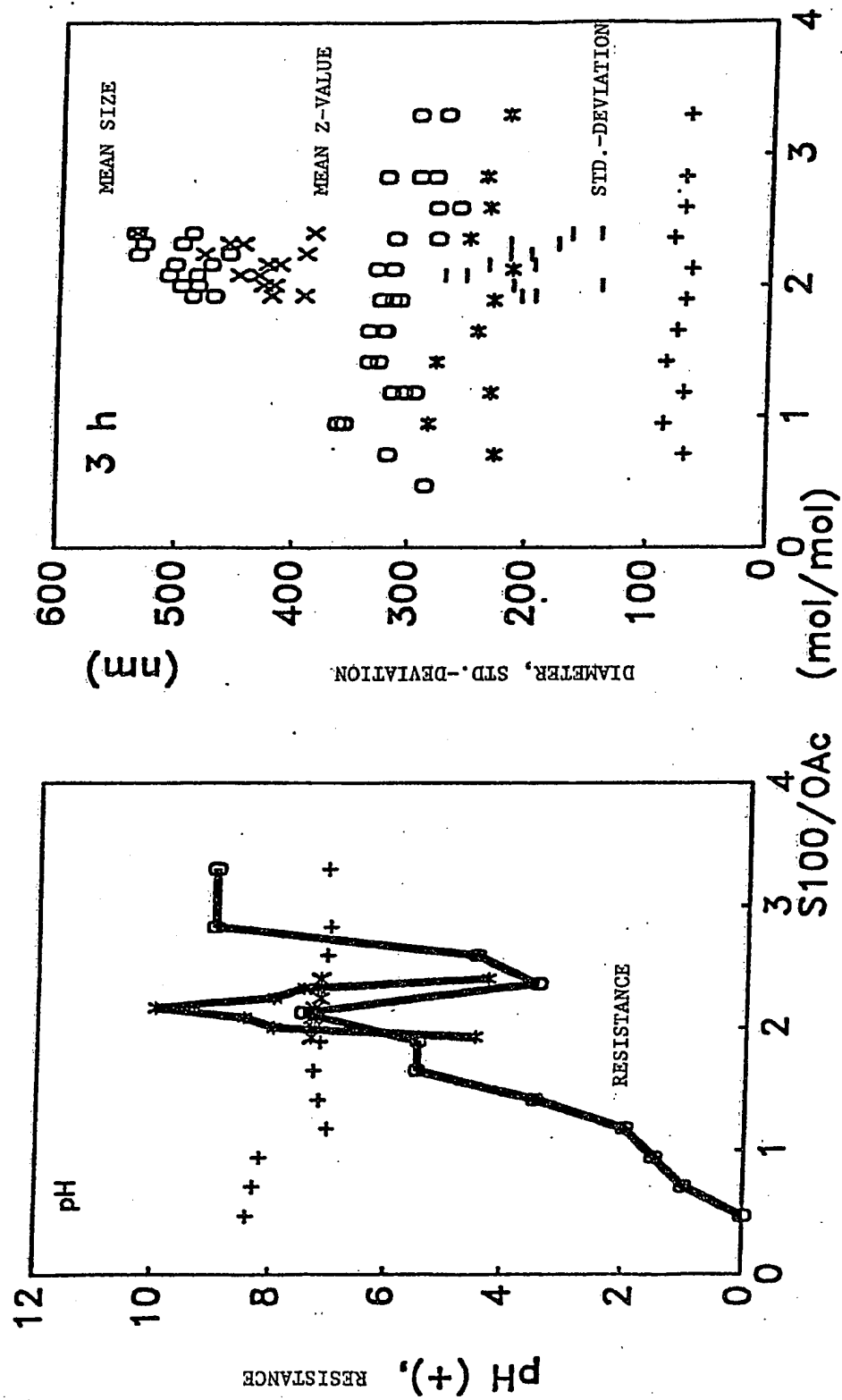
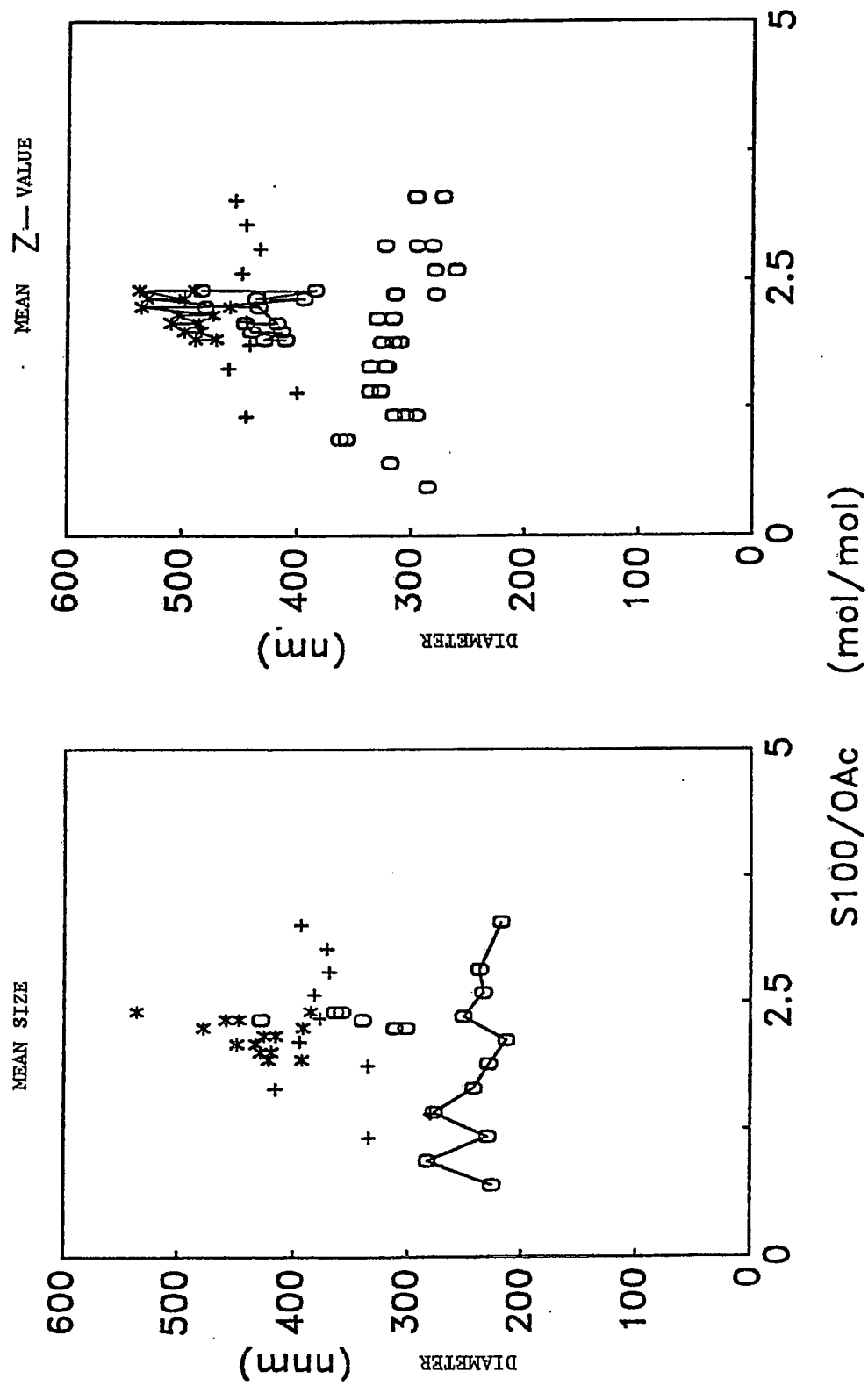


FIGURE 2



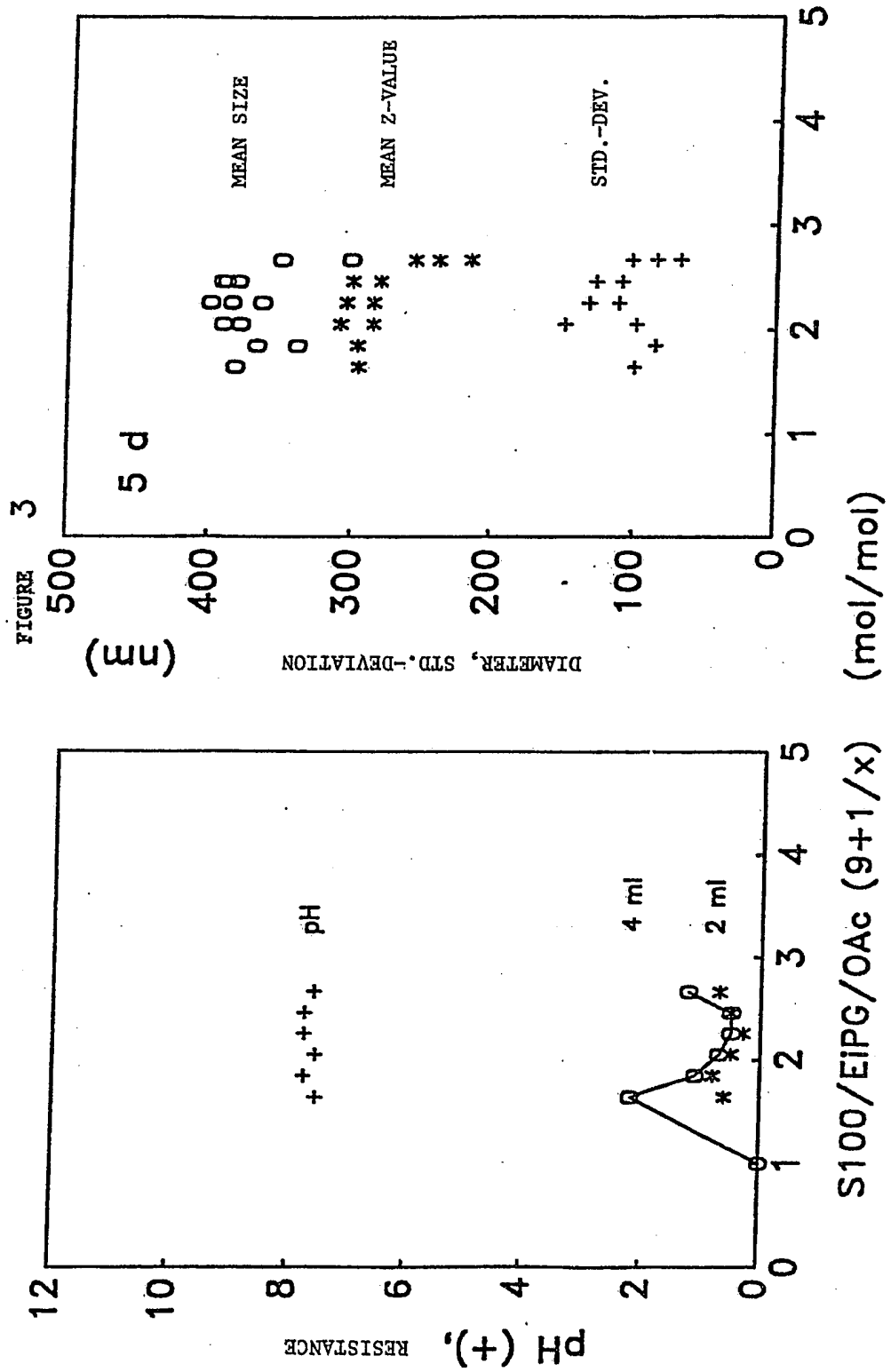


FIGURE 4

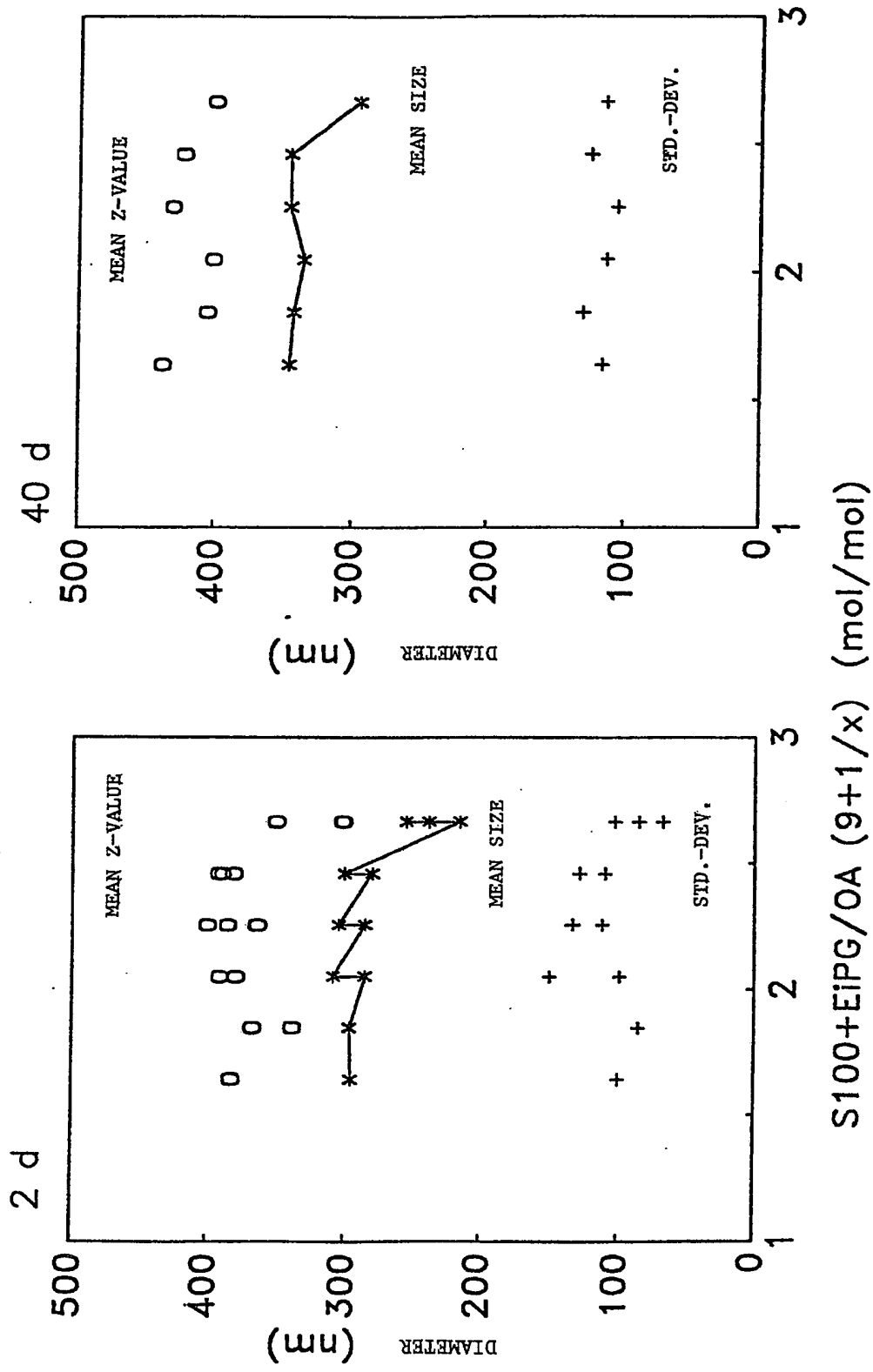


FIGURE 5

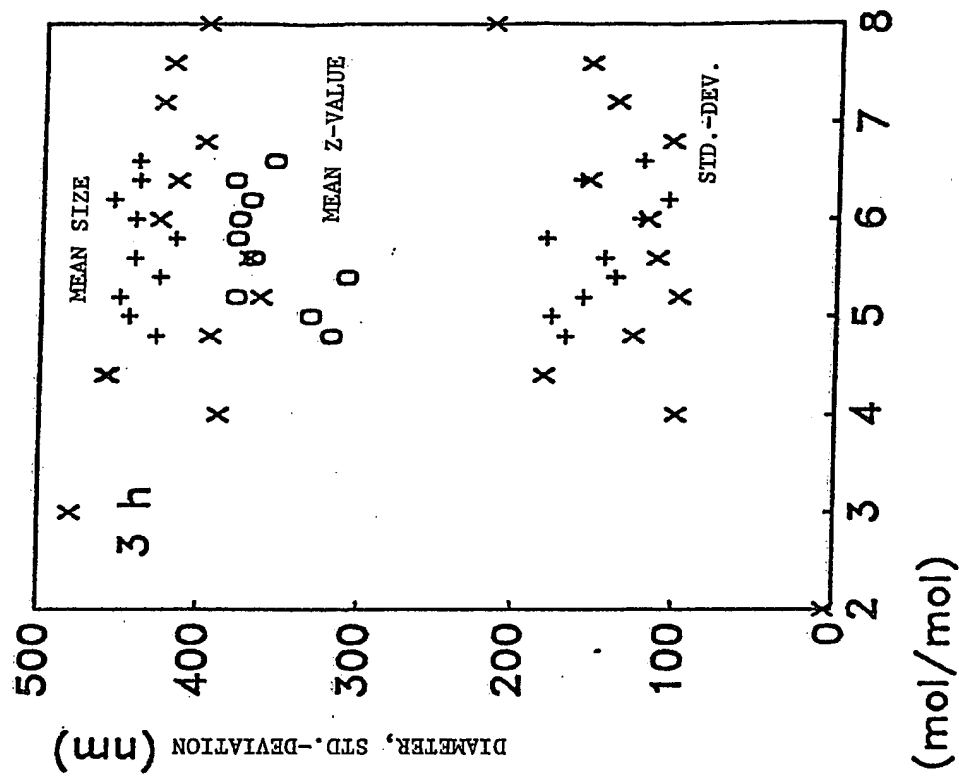
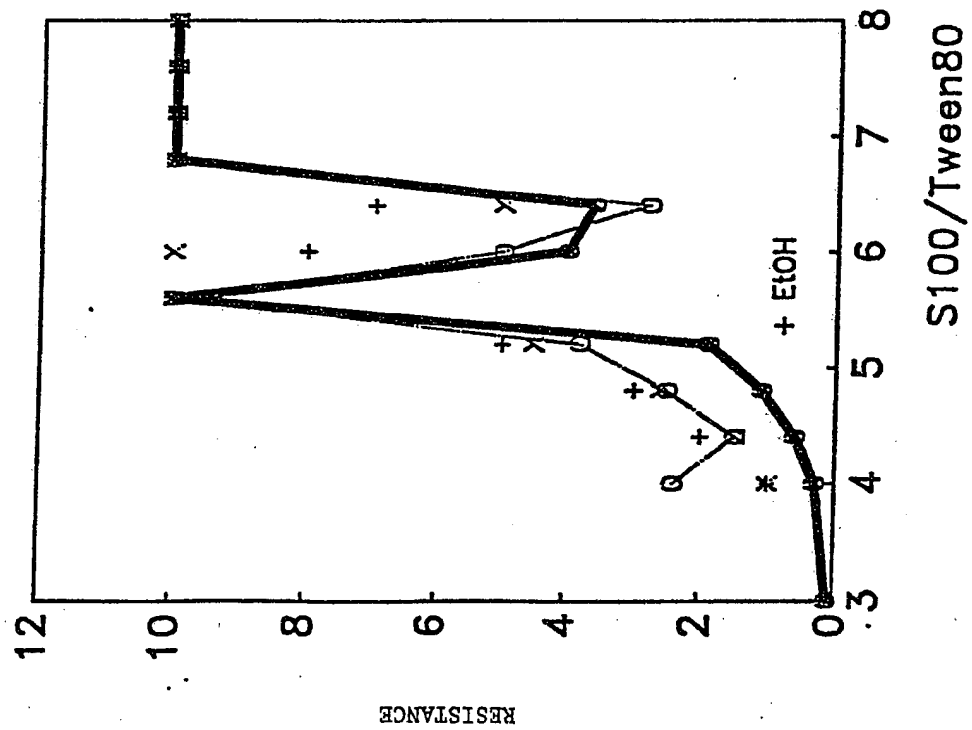


FIGURE 6

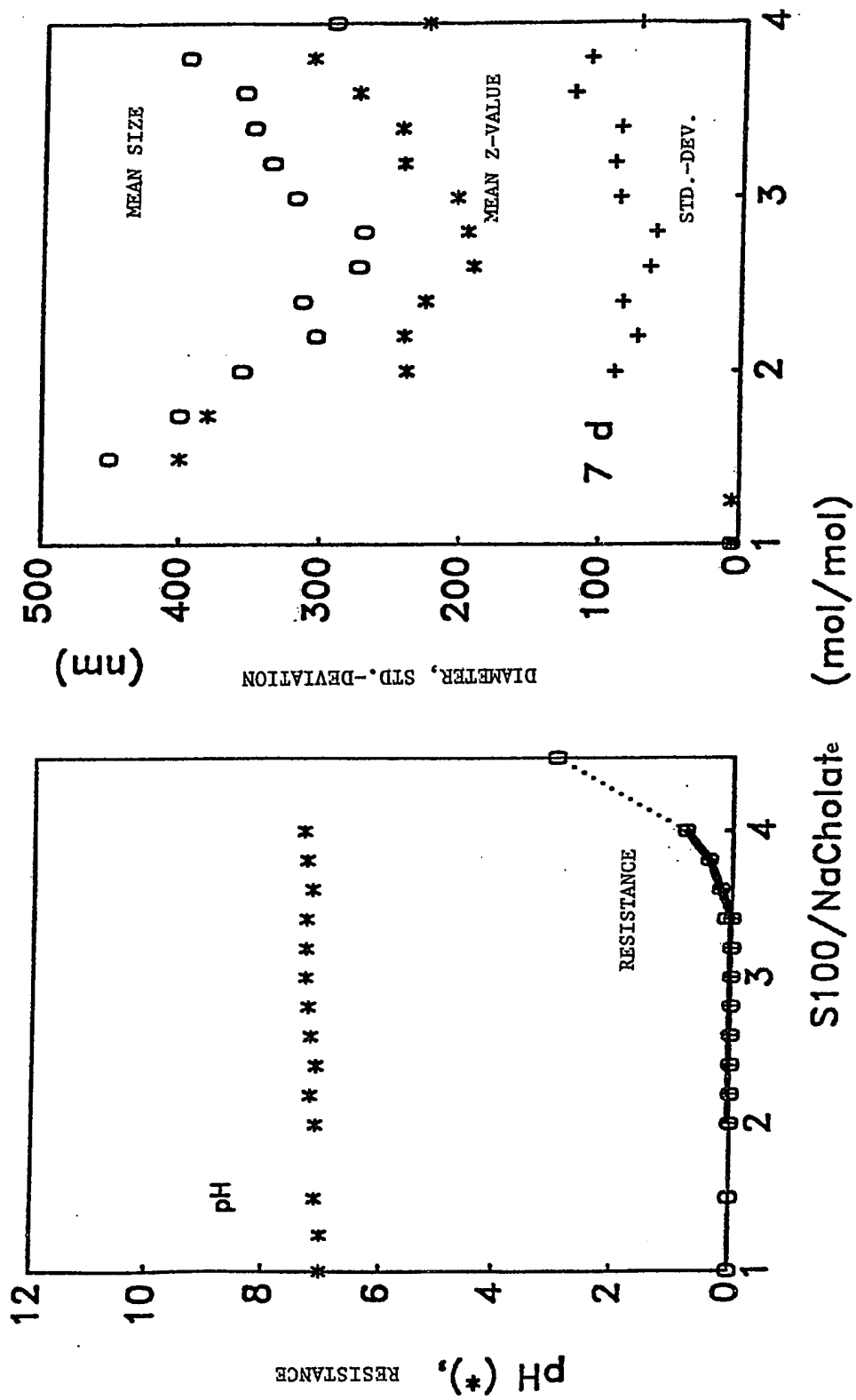
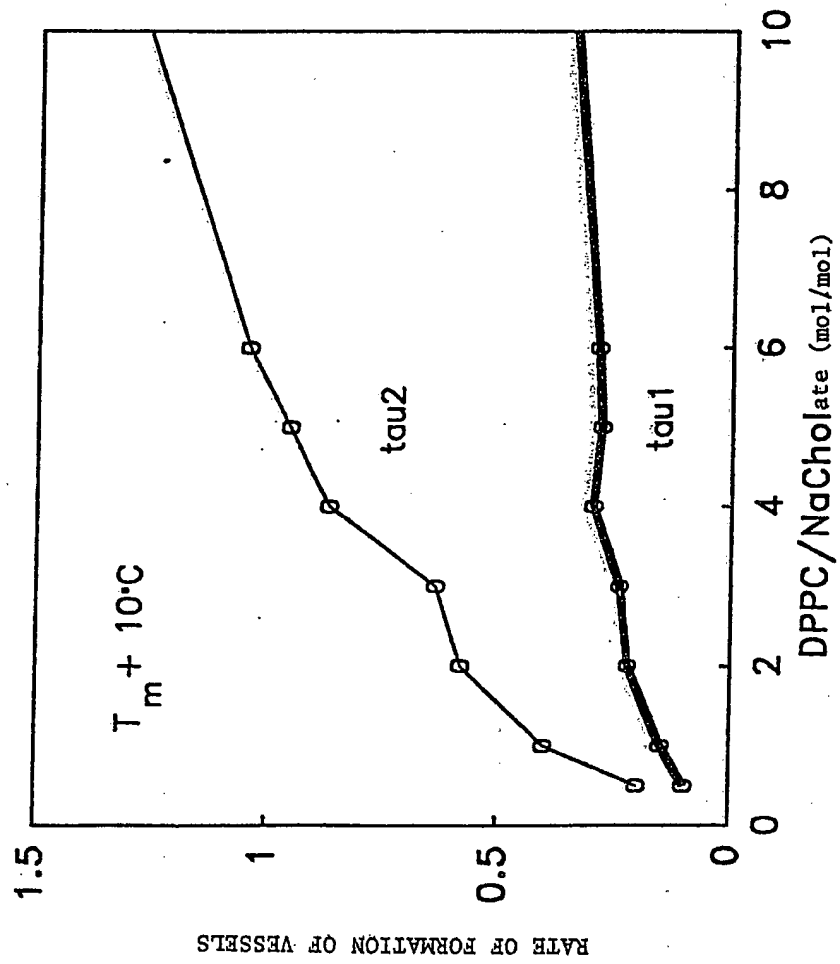
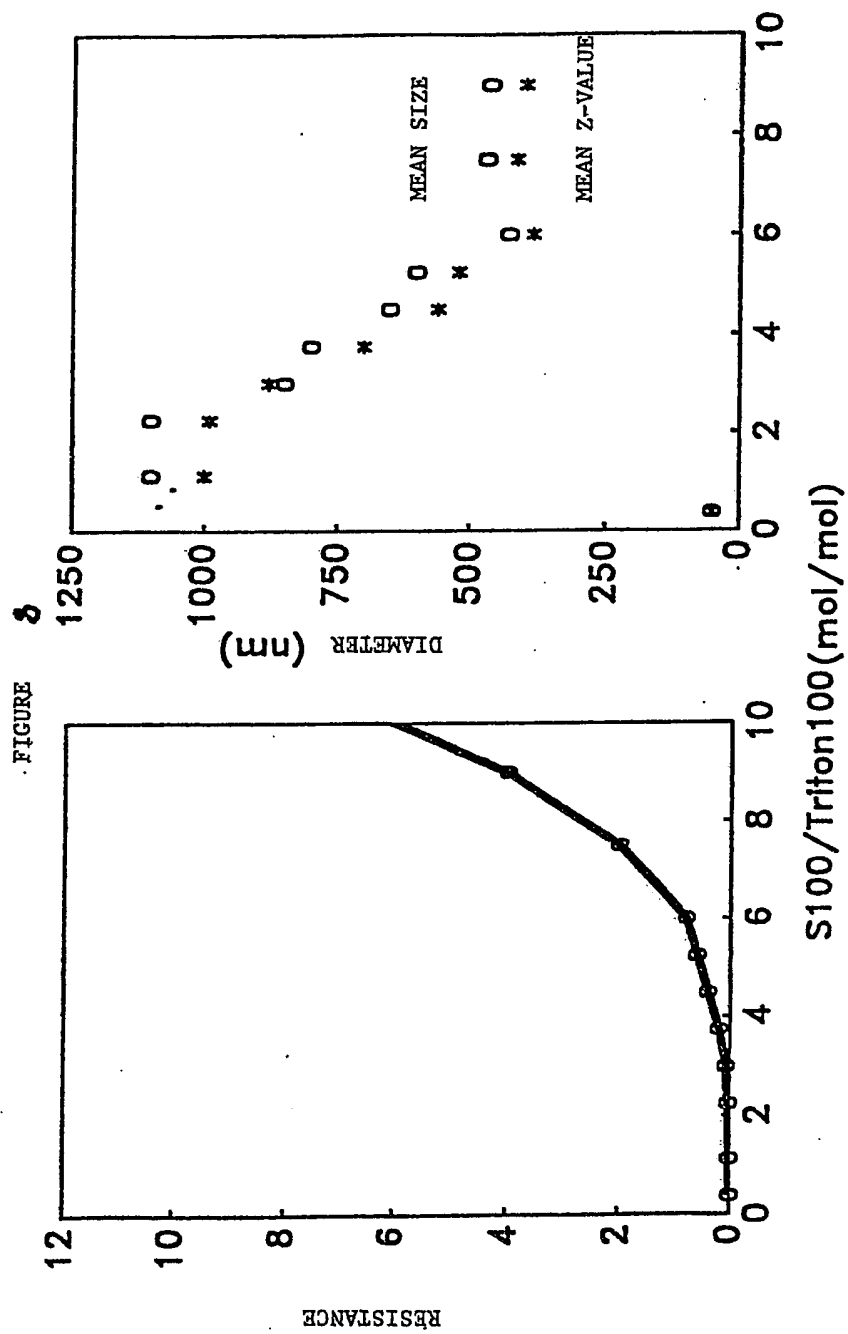


FIGURE 7





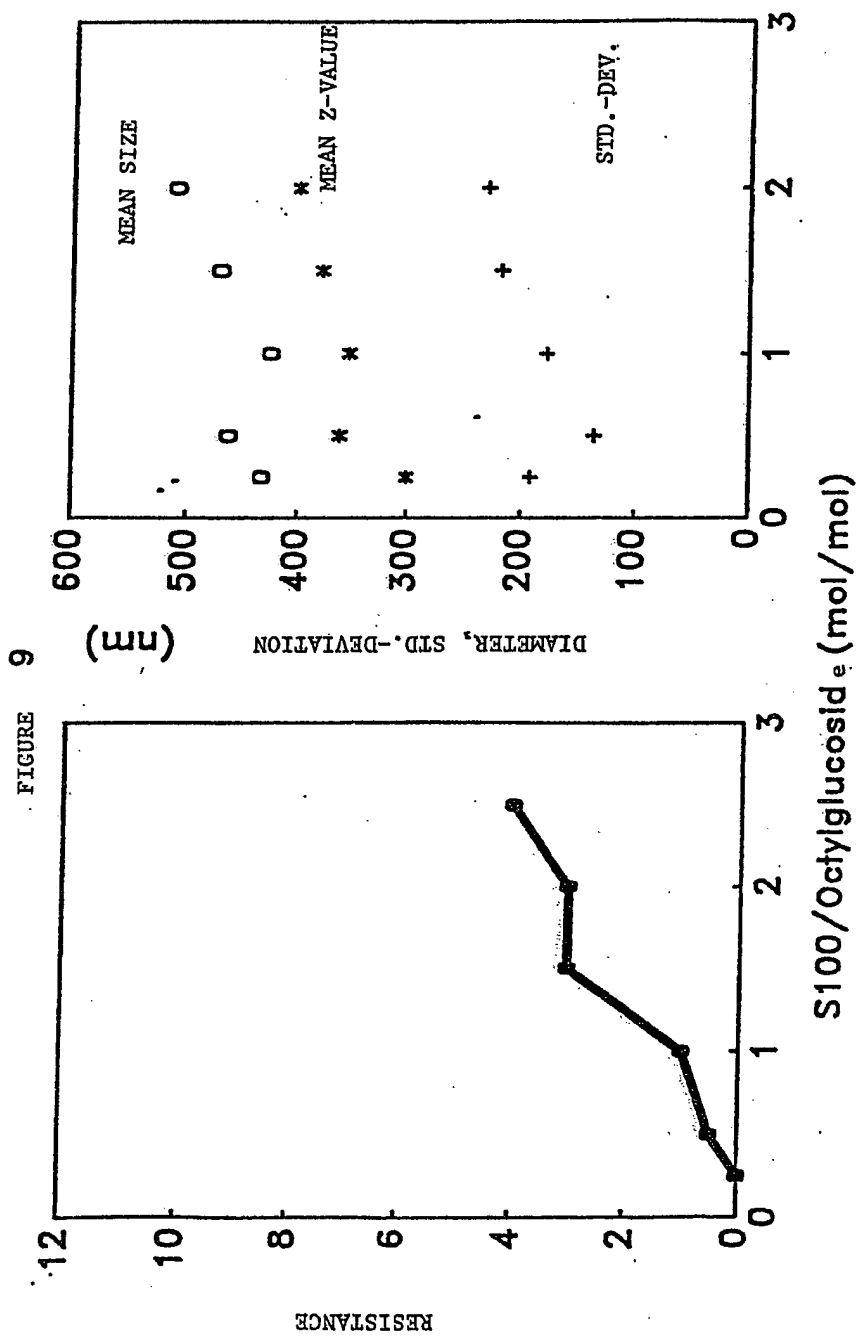
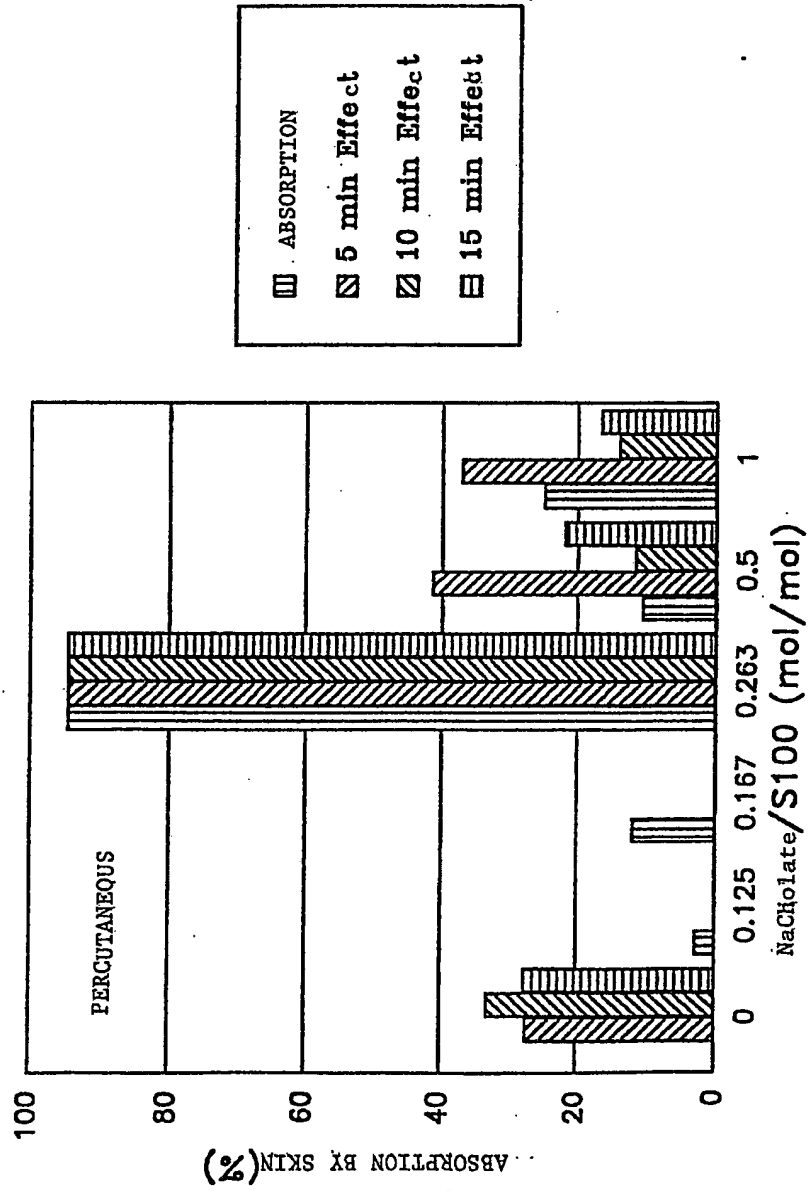


FIGURE 10



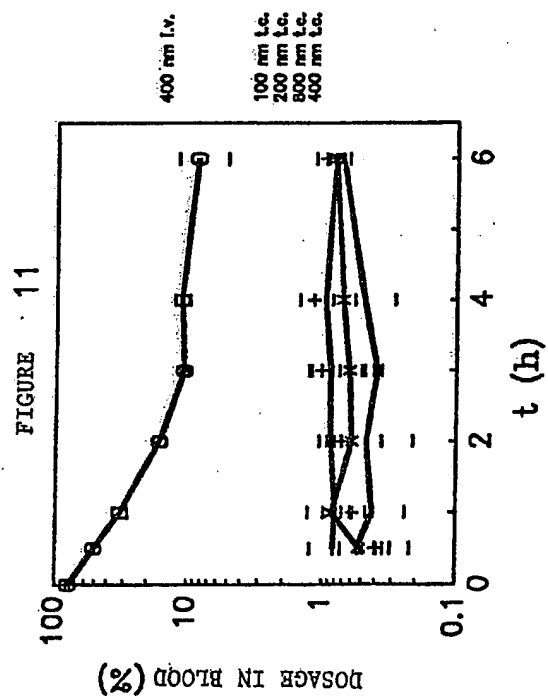


FIGURE 12

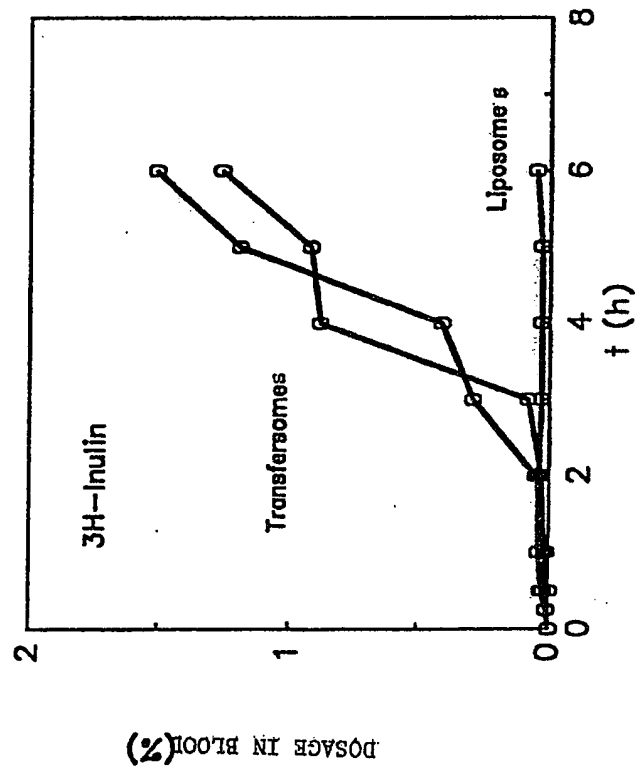


FIGURE 13

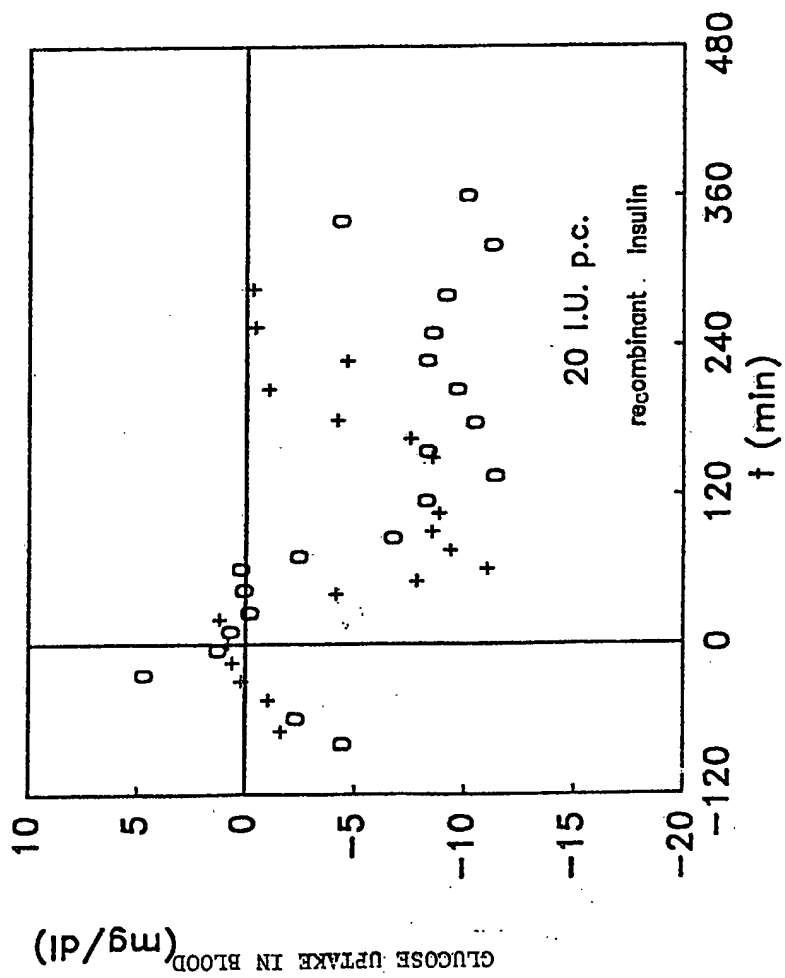


FIGURE 14

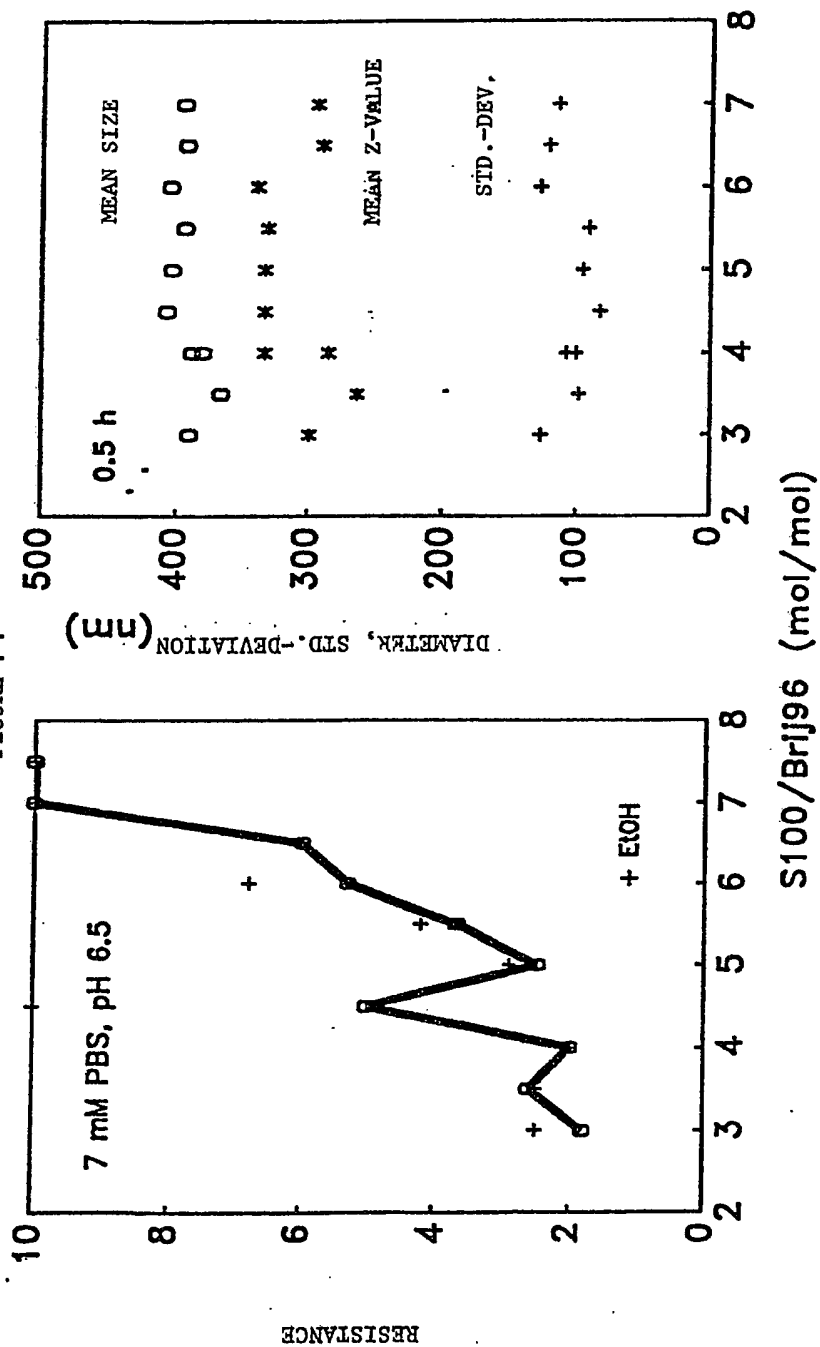


FIGURE 15

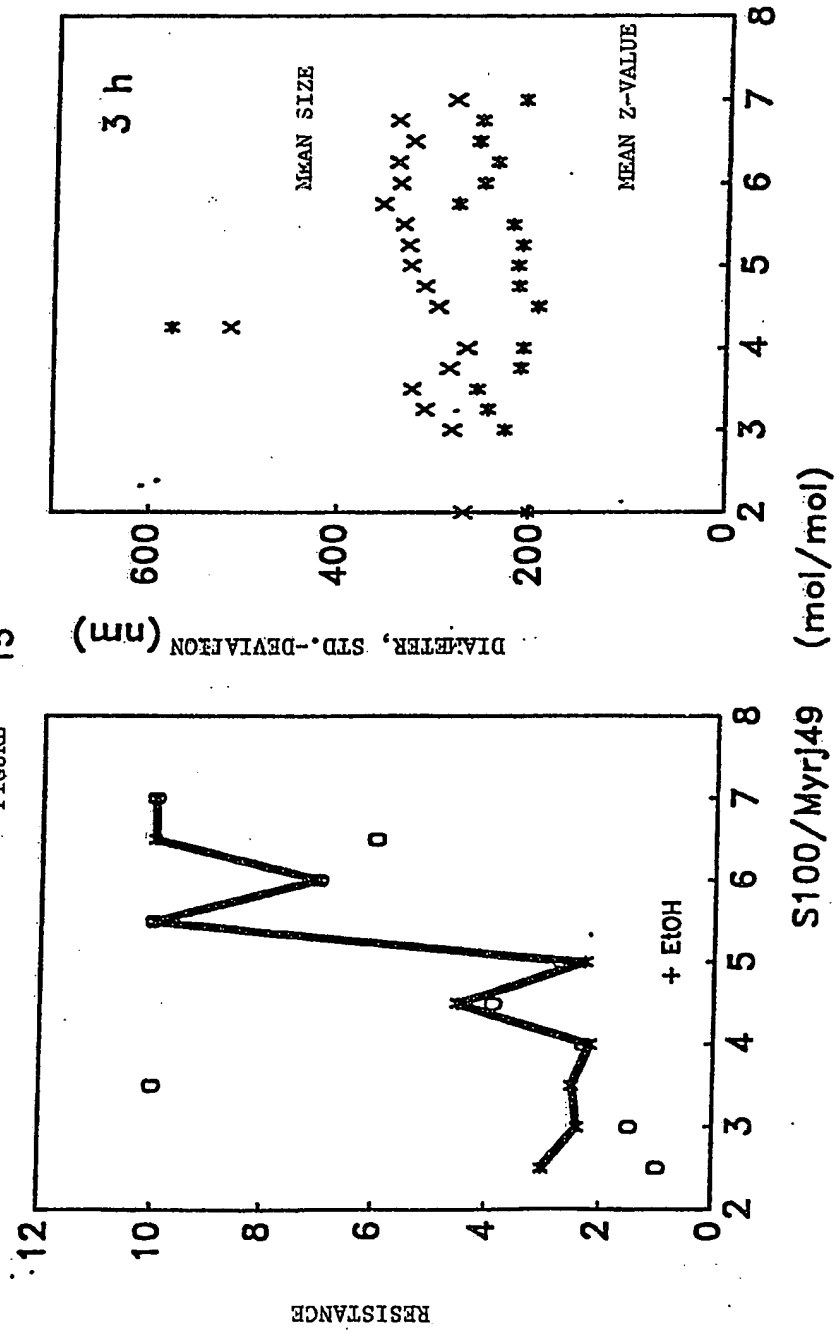


FIGURE 16

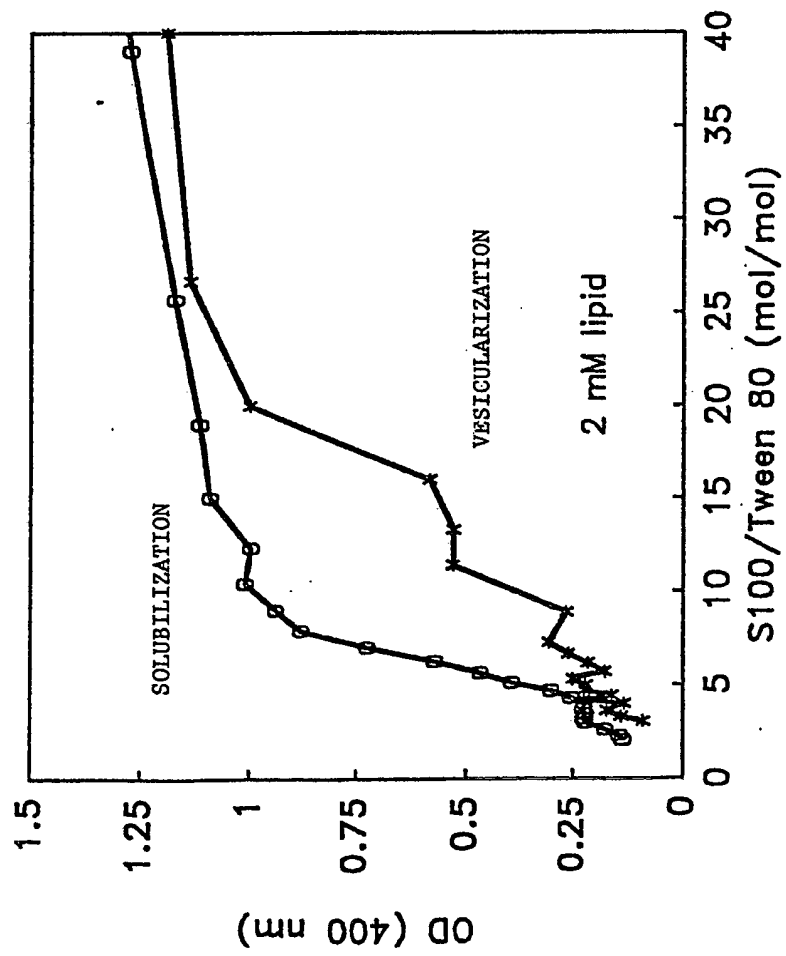


FIGURE 17

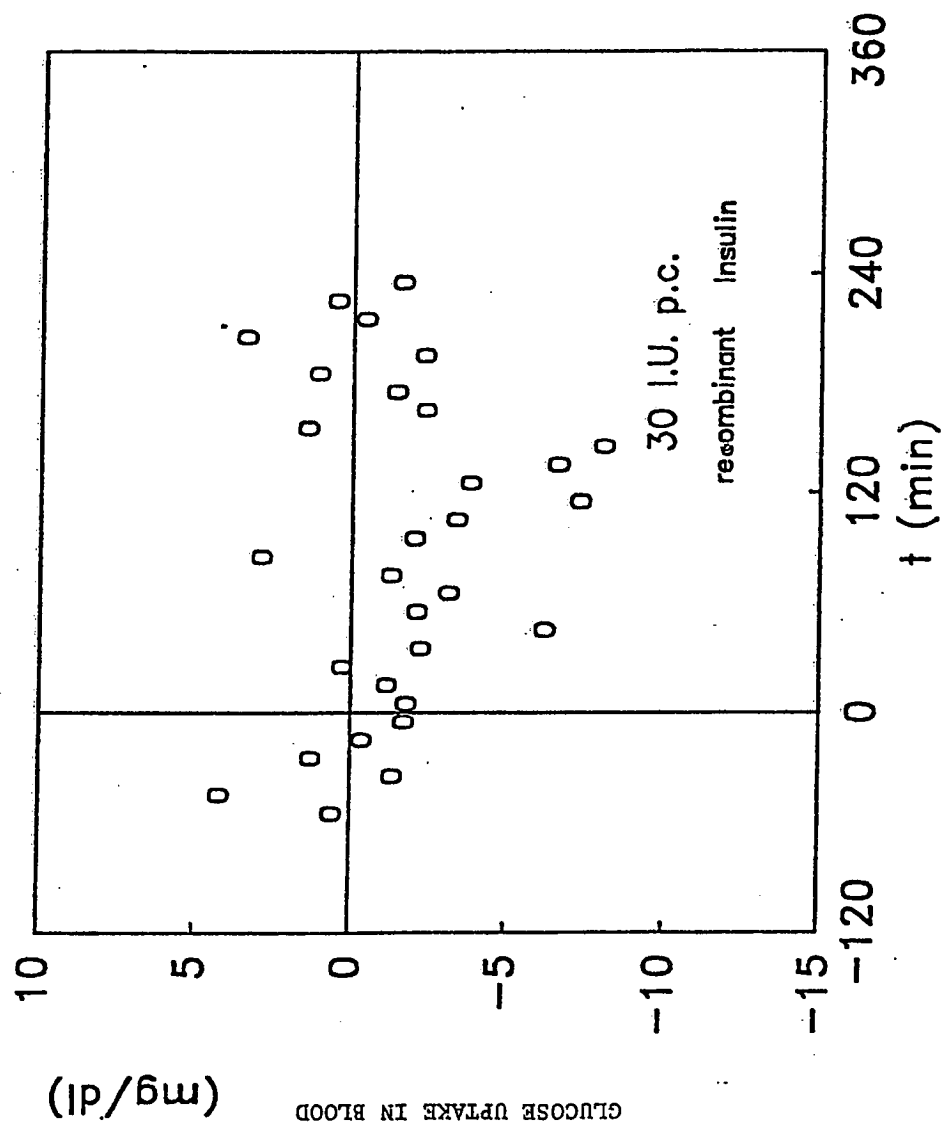


FIGURE 18

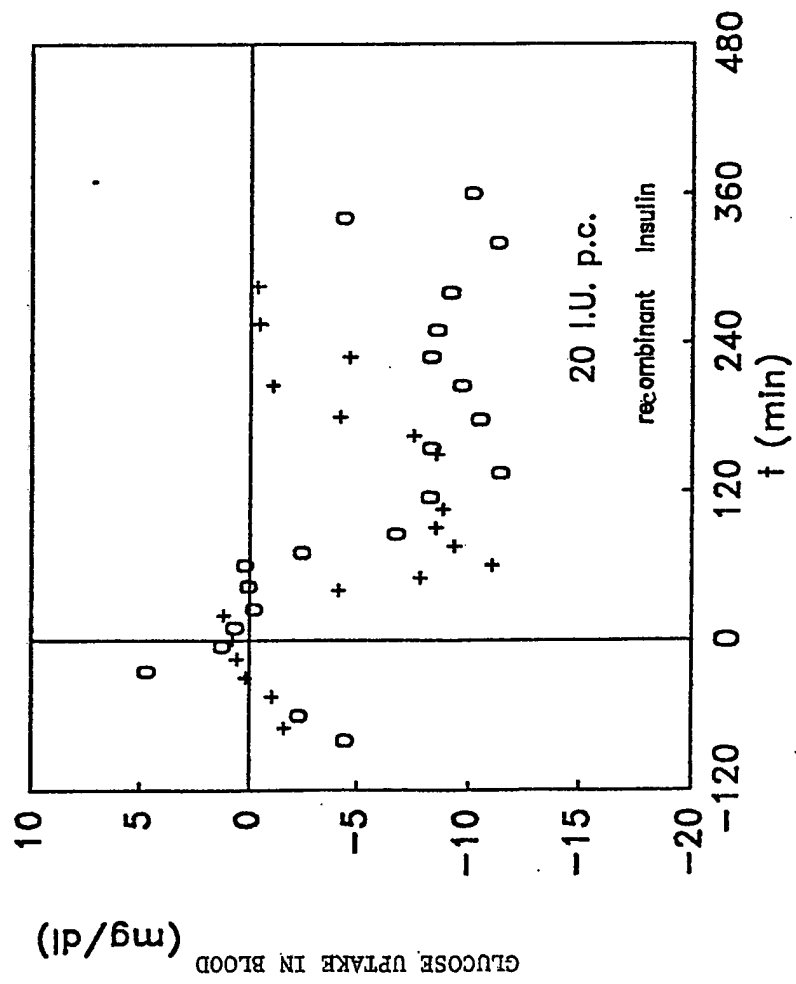


FIGURE 19

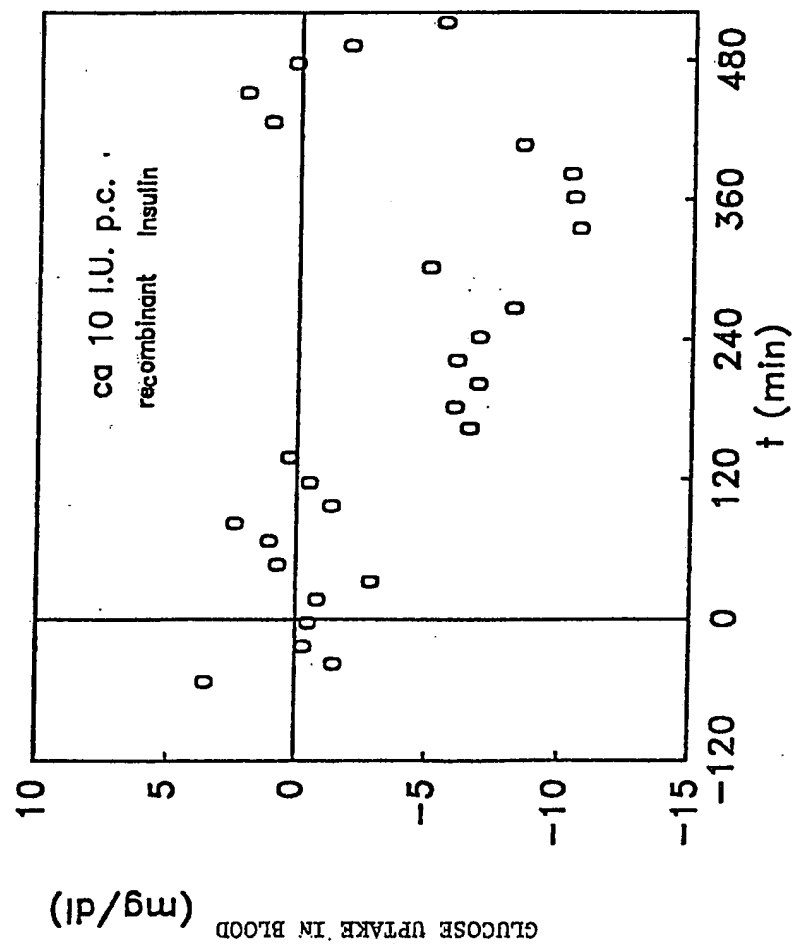


FIGURE 20

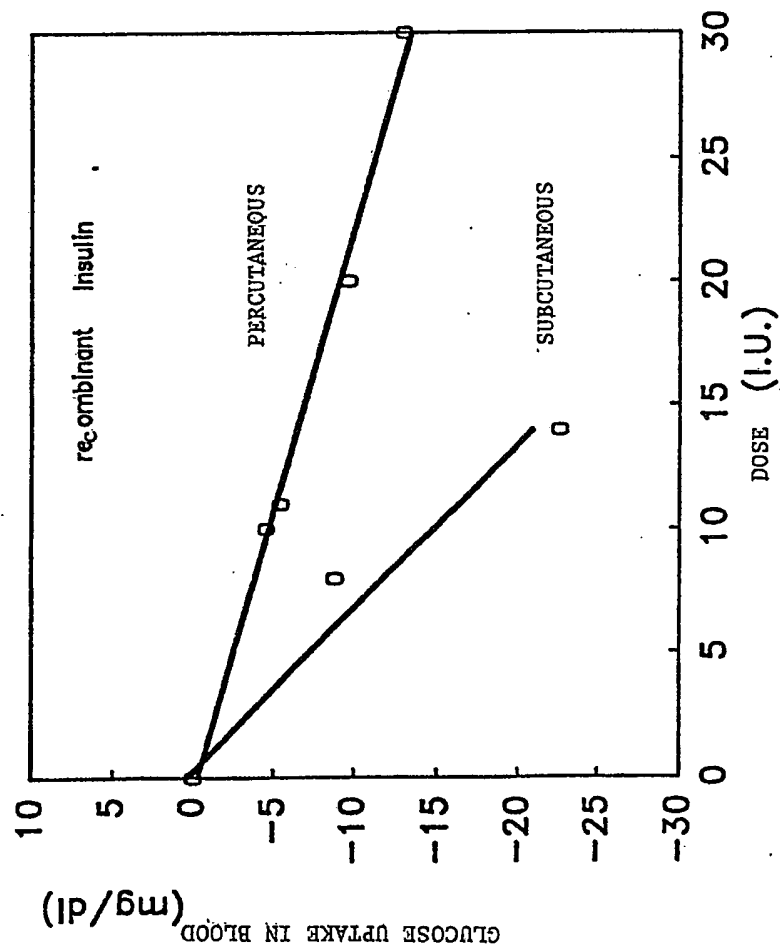
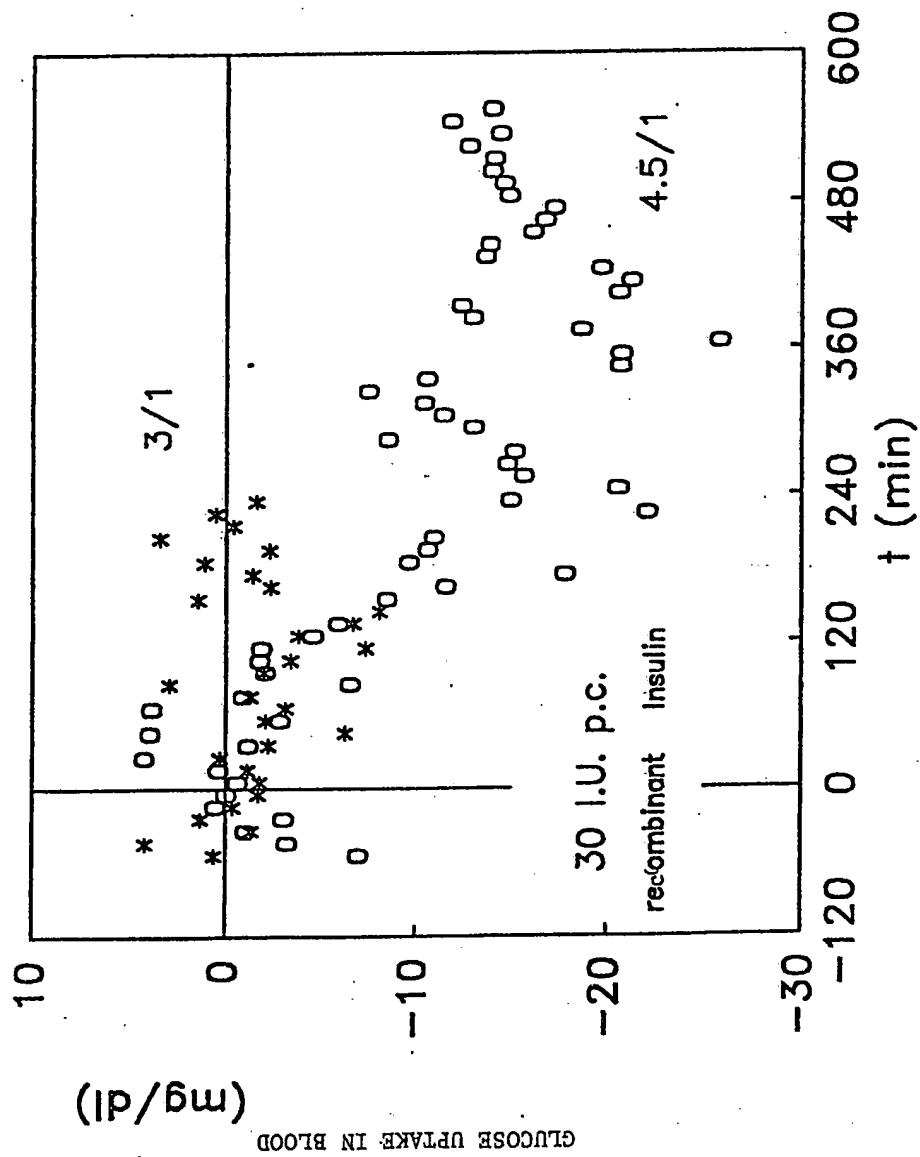


FIGURE 21



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